MOSQUITO ECOLOGY IN RELATION TO LAND-USE CHANGES AND POTENTIAL WEST NILE VIRUS IN NEWFOUNDLAND

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by

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ABSTRACT

The last extensive mosquito survey of insular Newfoundland was carried out in the early 1980s. Increased urban and agricultural development have locally increased the productivity of mosquito habitats and thus provided opportunity for colonization by additional species. The recent introduction of West Nile virus to North America and changing local climate has prompted renewed research on mosquitoes and the impact of human activity on them in Newfoundland. The research objective was to redefine species composition and evaluate the impact of land-use on mosquitoes. Data were obtained by a survey of three cities and two agricultural areas as well as natural habitat using a standard dip method for larvae and CO_2 baited miniature light traps for adults. A horse serum survey was performed to detect the presence of the virus through ELISA antibody screening, in addition to the National corvid surveillance program serving as an indicator for virus activity. Three additional mosquito species were recorded, including *Culex pipiens* L. (Diptera: Culicidae). The research provided the data needed to estimate the level of risk for human and animal exposure to West Nile virus. Such information will be essential if the risk of vectored disease transmission increases due to enhanced mosquito breeding seasons.

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1 GENERAL INTRODUCTION

The mosquito family (Diptera: Culicidae) contains over three and a half thousand species classified into three subfamilies: Toxorhynchitinae, Anophelinae, and Culicinae. The Anophelinae includes three genera, the Toxorhynchitinae includes a single genus, and the subfamily Culicinae has 34 genera. Culicinae is the largest and most diversified subfamily thereby being divided into ten tribes. This is the classification currently followed in *A Catalog of the Mosquitoes of the World* (Knight and Stone 1977) and its three supplements (Ward 1992, Gaffigan and Ward 1985, Ward 1984, Knight 1978). The genus *Aedes*, contained in one of the largest Culicinae tribes Aedini, has recently been divided into two genera on the basis of characteristics of the female and male genitalia (Reinhert 2000). This new classification for the genus *Aedes*, by elevation of subgenus *Ochlerotatus* to generic rank, has not been universally acknowledged, but for this thesis I have accepted the designation of these taxa following Reinhert (2000).

Fifteen genera characterize the mosquito fauna of North America, representing the three subfamilies; one Toxorhynchitinae, three Anophelinae, and 11 Culicinae from seven tribes (Darsie and Ward 1981). Mosquitoes are of interest because of their epidemiological significance as vectors of pathogenic organisms and as nuisance pests. Aspects of a mosquito's life history which inhibit or improve pathogen cycles, such as its breeding habitat, blood-feeding behaviour, and potential to increase in numbers, become important. Changes in composition of mosquito fauna and life history strategies in a given area can significantly impact the risk to human and animal populations. These changes, whether due to climate or land use, may render mosquito breeding and/or

pathogen replication more favourable than previous to the change. The Island of Newfoundland (106, 000 km^2) is primarily boreal forest habitat with peatlands being a particularly extensive and common type of wetland found in this ecozone (Wells and Pollett 1983). These habitats, as well as snowmelt pools, serve as primary breeding grounds for mosquitoes. The low productivity of lentic Newfoundland habitats, which is limited by low levels of nutrients, generally restricts aquatic insect faunal diversity (Larson and Colbo 1983). However, localized impacts of disturbance such as pollution, ranging from eutrophication to application of pesticides, and alteration of drainage systems and patterns may affect the composition of mosquito communities and the distribution of mosquito species due to these modifications of larval habitat. Historically, Newfoundland has had limited areas of land developed for agriculture or urbanization. Therefore marked recent expansion of these forms of land use provides a greater potential for changes in insect populations, depending on their ability to utilize these altered habitats. There are many studies that concentrate on the effects of anthropogenic disturbance on invasive mosquito species, or those of epidemiological significance, leading to artificial container breeding and increases in population size (Reiskind et al. 2004, Lounibos 2002, Andreadis et al. 2001, Beehler and Mulla 1995). There is little focus in the literature however about the transition of habitat from natural to disturbed and the potential subsequent change in the composition of mosquito fauna, particularly in a boreal ecozone.

1.1 Objectives

The objectives of this study were to (1) redefine mosquito species composition of insular Newfoundland, (2) evaluate the impact of land use, particularly urbanization and agricultural activities, on mosquitoes, and (3) estimate the level of risk for human and animal exposure to potential West Nile virus transmission in Newfoundland.

1.2 Mosquito life history

Like other true flies, mosquitoes exhibit complete metamorphosis and progress through seven stages of development, including egg, four larval instars, pupa, and adult. Larvae are restricted to aquatic environments, which are often small and shallow bodies of water with little or no water movement. These habitats can include shallow pools, sheltered stream edges, marshes, water-filled treeholes, plants, and artificial containers. Most species live in freshwater but a few have adapted to life in brackish or saline waters in salt marshes, rock pools, or inland saline pools (Clements 1992, Wood et al. 1979).

1.2.1 Larval respiration and feeding

Respiratory spiracles of culicine and toxorhynchitine larvae are situated at the end of a tube or siphon, at the end of the abdomen, and are open to the air as the larvae hang down from the water's surface. The spiracles of anopheline larvae are located on the dorsal surface of the last abdominal segment and therefore float parallel to the water surface, their spiracles opening through the air-water interface. Larvae of the Culicine genera *Mansonia* and *Coquillettidia*, remain permanently submerged and have modified siphons with spike-like structures that penetrate the tissues of freshwater macrophytes

from which they obtain oxygen. Mosquito larvae use modified mandibles and maxillae (labial brushes) to obtain food from the water surface, typical of anopheline larvae, or the water column and substrate, as seen with culicine larvae. Food resources consist of microorganisms, such as bacteria, diatoms, and algae, and also particles of detritus derived largely from decayed plant tissues. Toxorhynchitine larvae however, are predatory on small invertebrates, as are a few species in the other two subfamilies. Feeding does not occur during the pupal stage in which the pupa floats at the air-water interface, respiring through the mesothoracic spiracles which open within large 'respiratory trumpets', the rims of which protrude through the water's surface (Clements 1999, Clements 1992).

1.2.2 Reproduction and host-feeding patterns

In many species, mating occurs when females are seized in male aerial swarms. These assemblages are sometimes made at locations of biological significance, such as their emergent sites or around their vertebrate hosts (Clements 1999). In others, males may approach isolated, resting females. Varieties of stimuli are used in mating behaviour, including volatile sex pheromones and female tone or wing beat frequency. Males use their antennae to orient to the sound and to the pheromones (Clements 1999). Male and female mosquitoes use their proboscis to obtain nectar and other plant juices as a source of carbohydrates to provide energy for survival, routine metabolic maintenance, and flight (Clements 1992). In addition, most female mosquito species are anautogenous (i.e. require a large amount of protein contained in a vertebrate blood meal for egg development); while some species are autogenous and can use protein reserves

accumulated during the larval stages to produce usually only the first batch of eggs (Clements 1992, Wood et al. 1979). In contrast, toxorhynchitine females subsist only on plant nectar and are rarely known to take blood meals, as is the case with the culicine *Wyeomyia smithii* (Coquillett). Blood-feeding female mosquitoes use chemoreceptors on the antennae and palps which are stimulated by carbon dioxide and body odours to detect the presence of a host. The females respond by flying upwind towards the host and use visual cues, thermal stimuli, and moisture for orientation as they approach (Clements 1999).

Mosquitoes are often classified as generalists or specialists with reference to their host-feeding patterns. Generalists, or opportunistic feeders, feed freely on a wide variety of host types; whereas specialists feed mainly on primary hosts and tend to limit feeding to specific host types. It should be noted however that many factors govern the host-feeding pattern of mosquitoes including the innate tendencies of the mosquito that will determine its responsiveness to different potential hosts, the flight habits of the mosquito, the defensive behaviour of the potential hosts, and seasonal changes in host availability (Clements 1999). For the larger genera, limited information is known on host-feeding patterns of species; however, some generalizations can be made. Many *Anopheles* species feed exclusively on mammals, particularly significant to their medical importance in parasite transmission. It is suggested that mammals are also the primary hosts of most *Aedes/Ochlerotatus* species, while other species with opportunistic feeding behavior feed not only on mammals but also on birds and reptiles when available. The feeding patterns of only relatively few species of the large *Culex* genus are known. For some species the

primary hosts are mammals and for others birds, while certain species feed regularly on both mammals and birds. In addition, a small percentage of *Culex* species feed on amphibians or reptiles such as *Culex territans* Walker which feeds almost exclusively on frogs (Wood et al. 1979). Mammals are the primary hosts of some *Culiseta* species while others feed primarily on birds, a pattern that is also seen in the *Mansonia* and *Coquillettidia* genera (Clements 1999).

1.2.3 Oviposition

An engorged female flies from her host to a suitable site for digestion of the blood meal and egg development. If blood feeding is disrupted by the defensive responses of the host and the volume of blood consumed is below that necessary to initiate egg development, a second partial meal is taken within minutes, or a few hours at most, of the first (Clements 1999). If the blood meal volume is sufficient, host-seeking and biting behaviours are inhibited. Between taking a blood meal and laying their eggs females are relatively inactive, although they may feed on plant juices during that time. Mature eggs will induce preoviposition behaviour and visual and chemical cues are used to identify habitats suitable for oviposition and subsequent larval development. The duration of a gonotrophic cycle, the period of time between one blood meal and the next, is affected by ambient temperature, which determines the rates at which the blood meals are digested and the ovaries develop (Clements 1999). Gonotrophic cycles may be as short as two or three days under tropical conditions and can extend to weeks under temperate and northern region conditions. Within a few hours or a day after oviposition, a female is again receptive to host cues and able to begin another gonotrophic cycle, of which she

may complete several in her lifetime. Wood et al. (1979) stated that five gonotrophic cycles in one year are probably the most a mosquito can achieve in Canada. Less than five percent of four major mosquito species of insular Newfoundland were found to complete three gonotrophic cycles (Mokry 1984).

Eggs may be dropped individually to float on the water surface, as by females of *Anopheles*, or packed together to form a floating egg raft, as by *Culex*. Several species, particularly the floodwater *Aedes/Ochlerotatus*, deposit their eggs on moist substrate, often at the edge of a body of water or on an area of soil that will be flooded. In addition to submergence in water, practically all aedine eggs require a hatching stimulus such as a decrease in oxygen concentration as well as increases in temperature (Clements 1992). Embryonic development in the egg is also dependent on temperature. Generally within a few days to weeks, a fully formed larva develops within the egg and is capable of hatching. Those laid on the surface of the water hatch as soon as development is complete.

1.2.4 Overwintering

The life span of adult mosquitoes may be several weeks to months in temperate regions, especially if the species overwinters in the adult stage. A critical event in the life cycle of Canadian mosquito species is the initiation and termination of diapause, which makes it possible for the mosquito to survive the winter. Wood et al. (1979) stated that overwintering is accomplished by Canadian mosquitoes in three basic types, distinguished by the stage of the life cycle: egg, larva, or adult female. The first group of species overwinters in the egg stage. Eggs laid by *Culiseta morsitans* (Theobald) and

almost all species of *Aedes/Ochlerotatus* enter a required diapause and will hatch only after being subjected to several weeks of cold temperatures, thus hatching is closely related to snowmelt. All species of *Psorophora* and 15 species of *Aedes/Ochlerotatus*, including *Ochlerotatus atropalpus* (Coquillett), *Ochlerotatus canadensis* (Theobald), *Ochlerotatus cantator* (Coquillett), *Aedes cinereus* Meigen, and *Aedes vexans* (Meigen), are similar but they are also capable of additional generations during the same summer if their eggs have dried and been subsequently reflooded. The time of their first hatching in the spring is also much more varied than *Cu. morsitans* and other *Aedes/Ochlerotatus* species. *Anopheles walkeri* Theobald is also a member of this group, overwintering in the egg stage and hatching in late spring, although this species differs because eggs of the first generation are laid on permanent water, so that development proceeds immediately, and many generations overlap allowing all stages to be encountered (Wood et al. 1979).

In the second group of species, the larval stage enters diapause in preparation for overwintering. Further subdivision of this group includes two univoltine (one generation per year) species: *Coquillettidia* (*Mansonia*) *perturbans* (Walker) and *Wy. smithii*, and a multivoltine subgroup which includes *Anopheles barberi* (Coquillett), *Toxorhynchites rutilus* (Coquillett), *Aedes sierrensis* (Ludlow), *Culiseta melanura* (Coquillett), *Orthopodomyia alba*Baker, *Orthopodomyia signifera* (Coquillett), and southern populations of *Wy. smithii*. Species of the third group overwinter as inseminated nonblood-fed females. Two northern species, *Culiseta alaskensis* Ludlow and *Culiseta impatiens* (Walker), have an extremely long life span of a year or more. Newly emerged females do not seek a blood meal in their first summer and are therefore among the

earliest species to seek blood the following spring. Other members of this group undergo successive summer generations, resulting in overlapping generations, including *Anopheles earlei* Vargas, *Anopheles freeborni* Aitken, *Anopheles punctipennis* (Say), and *Anopheles quadrimaculatus* Say; three species of *Culiseta*, *Culiseta incidens* (Thomson), *Culiseta inornata* (Williston), and *Culiseta minnesotae* Barr; all species of *Culex*; and *Uranotaenia sapphirina* (Osten Sacken) (Wood et al. 1979).

1.3 Mosquitoes as arbovirus vectors and West Nile virus in North America

Their innate blood-sucking tendency renders adult mosquitoes prone to acquire microorganisms and parasites from one vertebrate host and to pass them on to another, but even so, many aspects of a mosquito's ecology and physiology must be appropriate for it to acquire, harbour, and transmit a particular pathogen. With respect to arboviruses (arthropod-borne viruses), they are naturally maintained in cycles by haematophagous (blood-feeding) arthropods that biologically transmit viruses between vertebrate hosts; that is, the pathogens undergo a propagative mode of development in the arthropod vector before being transmitted to the next vertebrate host. Arboviruses achieve long-term survival by maintaining ongoing transmission between vertebrate hosts and vectors, prolonging infections of a host or vector, and by employing overwintering mechanisms. As a result, during periods of little or no transmission, the number of infected hosts and vectors declines (Woodring et al. 1996). The length and number of gonotrophic cycles a particular mosquito species exhibits relates to the extent to which transmission may be taking place. If a vector experiences more than one gonotrophic cycle in its life span, its

potential to be an effective vector increases because it is more likely to transmit a pathogen to a greater number of vertebrate hosts and possibly its progeny.

A pathogen cycle enters a transeasonal maintenance phase through overwintering mechanisms and an opportunity for transmission ceases (Woodring et al. 1996). Several strategies or mechanisms have been proposed or observed that permit pathogen survival during these adverse conditions. The virus can persist in the adult vector when an adult remains dormant during the unfavourable season. For example, *Culex quinquefasciatus* Say infected with St. Louis Encephalitis (SLE) virus has been shown to survive under natural winter conditions and remain infected (Chamberlain and Sudia 1961) as well as *Culex pipiens* Linnaeus with SLE virus (Bailey et al. 1978) and West Nile virus (WNV) (Nasci et al. 2001). The egg may also serve as a reservoir for virus when vertical transmission occurs, in which a virus-infected female transmits the pathogen to her progeny transovarially (Woodring et al. 1996). A study by Watts et al. (1973) indicated that female mosquitoes infected with La Crosse virus can transmit this virus transovarially to their progeny and these progeny can transmit this virus by bite, thus initiating a new transmission cycle. Various studies also indicate that WNV (Dohm et al. 2002b, Baqar et al. 1993) and the closely related Japanese Encephalitis (JE) (Rosen et al. 1989, Rosen et al. 1978) and SLE viruses (Hardy et al. 1984, Francy et al. 1981) can be transmitted vertically by their mosquito vectors. This would allow female mosquitoes to become infected either transovarially or by ingesting a viremic blood meal in the late fall, survive the winter, and reintroduce the virus the next spring.

In the case of arboviruses, the vector provides a specific physical and chemical site that permits the pathogen to increase in numbers. Turell et al. (2002) outlined the several steps that must occur for a mosquito to transmit a virus by bite: (a) the mosquito must ingest a viremic blood meal; (b) the virus must then replicate in the midgut and 'escape' into the hemocoel, replicate throughout the body of the mosquito until it gets into the salivary glands, replicate in the salivary glands, and eventually be excreted into the saliva; (c) the infectious mosquito now must probe a susceptible host. In biological transmission of an arbovirus, the period between ingestion of an infectious blood meal and transmission capability or secretion of virus into the saliva, is known as the extrinsic incubation (EI) period (Turell et al. 2002, Woodring et al. 1996). In addition, the appropriate viral receptors must be present on the epithelial cells in the midgut of a mosquito, which ingests a viremic blood meal, for a viron to bind to that receptor and hence initiate the infection process (Turell et al. 2002). Therefore, there are multiple barriers a pathogen must overcome to be transmitted by the bite of a mosquito and these barriers will partially determine a vector's competence.

Turell et al. (2002) stated that if the proper conditions exist (i.e. temperature, mosquito species, mosquito population density, number of susceptible hosts, etc.), an epizootic outbreak of a disease will occur in a host population. Vector population size also plays an important role in disease transmission and will determine the number of contacts between vectors and susceptible hosts, a key factor in the epidemiology of a given disease. In addition, to transmit a pathogen successfully from one host to another, a mosquito must have a high probability of biting an infected host and in the case of

arboviruses, also must live long enough to bite another uninfected host after the EI period. Therefore longevity is very significant in determining how efficiently a mosquito species spreads a pathogen. Adult survival rate is one, if not the most important factor determining the stability of the population and total egg production because long-lived females can produce many eggs (Clements 1992). Thus, the longer a vector lives, the greater the probability of feeding after the EI period, resulting in more opportunities for transmission.

West Nile virus is a member of the flavivirus genus (Flaviviridae) which includes more than 70 distinct agents. WNV belongs to the Japanese encephalitis (JE) antigenic serogroup, as do SLE, JE, and Murray Valley encephalitis viruses (Mackenzie et al. 2002). All known members of this group are transmissible by mosquitoes and many of them can cause febrile, sometimes fatal, illnesses in humans. WNV was first isolated in 1937 from the blood of a febrile patient in the West Nile district of Uganda and was subsequently recognized as the most widespread of the flaviviruses, with a geographic distribution including Africa and Eurasia (Drebot et al. 2003, Hubálek and Halouzka 1999).

The first documented incursion of this virus into North America occurred during the summer of 1999 when an outbreak of neurological illness among humans, birds, and horses was identified in the New York City area (Nash et al. 2001, Anderson et al. 1999, Lanciotti et al. 1999). By 2002, WNV activity was documented in Quebec, Manitoba, Saskatchewan, and Nova Scotia and recursion occurred in Ontario. It has spread to all continental states of the US, excluding Alaska, and to date seven Canadian provinces (Nova Scotia, New Brunswick, Quebec, Ontario, Manitoba, Alberta, and Saskatchewan).

It is thought that the establishment of this pathogen within a certain area requires the presence of at least one primary mosquito species vector of WNV. In general, *Culex* species mosquitoes serve as vectors and passerine birds are vertebrate reservoirs in enzootic WNV transmission cycles. Since the New York outbreak in 1999 until 2001, WNV was recovered from 27 mosquito species in North America, including *Cx. pipiens*, *Culex restuans* Theobald, *Culex salinarius* Coquillett, *Oc. canadensis, Ochlerotatus japonicus* (Theobald), *Ae. vexans,* and *Cs. melanura* (CDC 2000, CDC 2001). To date, 60 mosquito species have been found in West Nile positive mosquito pools in the United States since 1999.

In Canada, 20 arboviruses have been found in either arthropod or vertebrate hosts, eight of which are known to cause disease in humans (Artsob 1990). Each is maintained in an enzootic amplification cycle of transmission between its mosquito or tick hosts and a range of wild birds and/or mammals that are normal vertebrate hosts and reservoirs, which again infect subsequent feeding mosquitoes or ticks. Periodically these viruses are transmitted by their arthropod vectorss to animals that are not their normal vertebrate hosts. For example, 'spillover' of the WNV from the bird-mosquito cycle to other hosts, such as horses or people, can occur. These hosts do not develop sufficient viremia to infect mosquitoes and therefore do not play a role in virus amplification and are considered to be incidental or "dead-end" hosts. Recent vector competence studies indicate that some North American *Culex* and *Ochlerotatus* species are relatively efficient laboratory vectors (Turell et al. 2001, Sardelis et al. 2001). In addition, mosquito species that are general feeders, and take blood meals from birds and mammals, likely act as the principal 'bridge vectors' of WNV, transmitting the virus to incidental hosts (Turell et al. 2002, White et al. 2001).

1.4 Mosquitoes of Canada

The mosquito fauna of Canada is characterized by ten genera that include six *Anopheles*, one *Toxorhynchites*, 47 *Aedes/Ochlerotatus*, five *Culex*, seven *Culiseta*, one *Coquillittidia*, two *Orthopodomyia*, four *Psorophora*, one *Uranotaenia*, and one *Wyeomyia* species. The mosquito fauna of Newfoundland and Labrador is dominated by widespread boreal species, most of which have island-wide distributions, although six of the previously recorded 30 species of the province were exclusive to Labrador, including one member of *Anopheles*, and two *Culex*, which were rarely found (Colbo 2003). Twenty-one *Aedes/Ochleratatus*, five *Culiseta*, and one *Wyeomyia* species were also recorded in the province (Colbo 2003, Nielsen and Mokry 1982b). The larvae of three species of mosquitoes on the island live in specialized habitats; these are *Wy. smithii*, which inhabits the water-filled leaves of pitcher plants, *Oc. cantator*, recorded to be found in saline rock pools along the coast, and *Oc. atropalpus* found in rock pools along rivers. Other species commonly found in the Maritimes include two *Anopheles*, eight *Aedes/Ochlerotatus*, two *Culex*, and *Cq. perturbans* (Wood et al. 1979). In addition, a recently introduced mosquito species in Canada is *Oc. japonicus,* which has become established in southern Ontario and Quebec (Darsie 2002). This species was introduced to North America from east Asia and was first detected in Connecticut and New Jersey, US, in 1998 (Andreadis et al. 2001, Peyton et al. 1999) and has since quickly extended its range.

1.5 Vector dispersal and land-use influences

There are several mosquito life history types, however larval habitats are concentrated in areas of permanent water or natural or artificial containers. Several environmental factors, including human activities, enhance vector population densities by creating such habitats. The formation of ditches, impounded water pools, agricultural land with increased nutrients, and standing containers such as tires, form niches enabling the mass breeding of mosquitoes (Mitchell 1996). An increase in these areas affects the distribution and abundance of mosquitoes thereby increasing the probability of a disease outbreak, assuming that an appropriate vector species is present; a widespread, abundant mosquito population needs to be in place to support epidemic expansion.

Turell et al. (2002) stated that the recent spread of JE virus into Asia and Australia has probably been through natural mosquito-vertebrate host cycles, possibly assisted by changes in land use. In fact, the potential of these habitats as opportunities waiting to be colonized by mosquito species is clearly recognized. For example, Day (2001) showed that dry conditions from mid-September through to the end of a year resulted in increased mosquito breeding in open sewage ditches, which provided an excellent source of organic-rich water in which *Culex* vectors of SLE virus could oviposit. Not only do transient water bodies, those that form in tree cavities or seasonal waterways for instance, provide an opportunity for breeding that might not otherwise exist in a dry region, but even in moist and temperate areas, they offer the further advantage of helping mosquitoes evade predators. Urbanization often leads to the lack of proper drainage of surface water,

resulting in an increase in vector breeding, an increase in human-vector contact, and an increase in pathogen transmission.

In recent centuries, human travel, trade, and development have repeatedly introduced species to new habitats and, for that matter, created entirely new habitats. An increase in larval habitats may not only alter mosquito populations already present, but also create a potential for habitat-specific mosquitoes, or opportunistic species, which may not have previously colonized an area, to establish (Lounibos 2002, Peyton et al. 1999). A common example is the highly publicized invasion of the Asian tiger mosquito, *Aedes albopictus* (Skuse), facilitated by tire shipments to the US. This treehole-breeding species spills over into artificial containers (Lounibos 2002) which offer a perfect counterpart to its natural habitat. An abundance of these habitats has allowed the establishment of this and several other species, such as the previously mentioned *Ochlerotatus japonicus* (Lounibos 2002, Peyton et al. 1999).

Aedes albopictus has been found infected with Eastern Equine Encephalitis (EEE) (Mitchell et al. 1992), LaCrosse encephalitis (Gerhardt et al. 2001), and WN viruses (Turell et al. 2002) since its establishment in North America. *Aedes albopictus* is a known vector of Dengue virus in its native range and has been found infected in Mexico and Brazil (Lounibos 2002), possibly playing a role in transmission, in addition to being a bridge vector for Sylvan yellow fever, and WNV (Turell et al. 2002). Evidently, the introduction of non-native mosquito species and the availability of a suitable habitat could render their pathogen-transmitting capabilities of significance to human populations if proper conditions, such as high population density, were sustained.

Furthermore, high population density will also permit some vectors that are otherwise poor hosts to a pathogen to be of significance. Hubálek and Halouzka (1999) report that in Africa and the Middle East, the main vector of WNv is *Culex univittatus* Theobald, in Europe the principle vectors are *Cx. pipiens, Culex modestus* Ficalbi, and *Coquillettidia richiardii* (Ficalbi), and in Asia, *Cx. quinquefasciatus, Culex tritaeniorhynchus* Giles, and *Culex vishnui* Theobald predominate. This shows that mosquito species are principle vectors in relation to their respective geographical region and their population abundance within these regions, thereby contributing to the proper conditions necessary for an epizootic outbreak of a disease to occur in a host population.

The last extensive mosquito survey was performed on the island of Newfoundland in 1981, including portions of the island which had not previously been systematically collected, such as the Northern Peninsula (Nielsen and Mokry 1982b). During this survey, four unreported species were found for the first time, *Ochlerotatus diantaeus* Howard, Dyar and Knab, *Ochlerotatus hexodontus* Dyar, *Ochlerotatus pionips* Dyar, and *Cs. melanura*. These species were further to the reporting of three additional species, *Ae. atropalpus*, *Ochlerotatus decticus* Howard, Dyar and Knab, and *Ochlerotatus sticticus* (Meigen). Insular Newfoundland's mosquito fauna was poorly known until this time and only four publications were available with information concerning the species on the island (Nielsen and Mokry 1982b). A checklist of species known to occur on the island up to this date included 19 *Aedes/Ochlerotatus*, three *Culiseta* species, and *Wy. smithii*. Since this survey, several other species have been added including *Culex* and *Anopheles* species; refer to Table 1.1 for a complete species list.

Table 1.1 A list of previously collected mosquito species in Newfoundland and Labrador and their first recordings.

	Previous collection locations	First Record
Aedes cinereus Meigen	Island-wide; Labrador	Wood et al. (1979)
Aedes vexans (Meigen)	Labrador	Colbo, MUN collection
Anopheles earlei Vargas	Labrador	Haufe (1952)
Culex restuans Theobald	Avalon Peninsula	Mokry, MUN collection
Culex territans Walker	Labrador	Freeman (1952)
Culiseta alaskensis Ludlow	Labrador	Haufe (1952)
Culiseta impatiens (Walker)	Island-wide; Labrador	Freeman (1952)
Culiseta melanura (Coquillett)	Near St. John's, Avalon Peninsula	Nielsen and Mokry (1982a)
Culiseta minnesotae Barr	Near St. John's, Avalon Peninsula	Mokry, MUN collection
Culiseta morsitans (Theobald)	Island-wide; Labrador	Pickavance et al. (1970)
Ochlerotatus abserratus (Felt and Young)	Island-wide; Labrador	Freeman (1952)
Ochlerotatus atropalpus (Coquillett)	Grand Falls, Burin Peninsula, Labrador	Nielsen and Mokry (1982b)
Ochlerotatus canadensis (Theobald)	Island-wide; Labrador	Freeman (1952)
Ochlerotatus cantator (Coquillett)	Avalon, Burin, and Northern Peninsulas; Labrador	Vockeroth (1954)
Ochlerotatus communis (DeGeer)	Island-wide ; Labrador	Wood et al. (1979)
Ochlerotatus decticus Howard, Dyar and Knab	Avalon Peninsula; Northern Peninsula; Labrador	Nielsen and Mokry (1982b)
Ochlerotatus diantaeus Howard, Dyar and Knab	Near Plum Point, Northern Peninsula; Labrador	Nielsen and Mokry (1982b)
Ochlerotatus excrucians (Walker)	Island-wide; Labrador	Freeman (1952)
Ochlerotatus fitchii (Felt and Young)	Island-wide, excluding Northern Peninsula; Labrador	Freeman (1952)
Ochlerotatus flavescens (Muller)	Labrador	Haufe (1952)
Ochlerotatus hexodontus Dyar	Hare Bay and Pistolet Bay, Northern Peninsula; Labrador	Nielsen and Mokry (1982b)
Ochlerotatus impiger (Walker)	Labrador	*
Ochlerotatus implicatus Vockeroth	Southwestern Region; Labrador	Wood et al. (1979)
Ochlerotatus intrudens Dyar	Avalon Peninsula; Labrador	Pickavance et al. (1970)
Ochlerotatus nigripes (Zetterstedt)	St. Anthony, Northern Peninsula; Labrador	Vockeroth (1954)
Ochlerotatus pionips Dyar	Northern Peninsula; Labrador	Nielsen and Mokry (1982b)
Ochlerotatus pullatus (Coquillett)	Northern Peninsula; Labrador	Vockeroth (1954)
Ochlerotatus punctor (Kirby)	Island-wide; Labrador	Freeman (1952)
Ochlerotatus sticticus (Meigen)	Little Power Pond, Avalon Peninsula; Labrador	Nielsen and Mokry (1982b)
Ochlerotatus stimulans (Walker)	Logy Bay, Avalon Peninsula	Pickavance et al. (1970)
Wyeomyia smithii (Coquillett)	Island-wide; Labrador	Freeman (1952)

Note: Reporting is based on species first recorded on insular Newfoundland, except those species exclusive to Labrador.
* Wood et al. examined specimens of this species, although for this purpose, previous literature could not be found.
MUN (Memorial University of Newfoundland).

Limited data on mosquito populations are available for the western portion of the province, an area of interest when considering potential species introductions from the mainland. Furthermore, the recent introduction of West Nile virus to North America has prompted renewed research on mosquitoes and the impact of human activity on them in Newfoundland. Changes in large expanses of land in the province for agriculture and urbanization can greatly affect the amount of nutrients entering mosquito breeding habitats and their distribution, thus having the potential to change the mosquito community. This change is of particular interest to people and domestic animals because of their proximity to breeding habitats and the threat of viral transmission. A changing climate may also compound this situation further by influencing mosquito species' distribution, establishment, and vector capabilities in addition to land-use alterations.

2 GENERAL METHODOLOGY

2.1 Study area

A comparison of mosquito communities influenced by agricultural and urban land-use was conducted in the primary area of study of insular Newfoundland with particular focus on St. John's (46°37'N, 52°45'W) and Deer Lake (49°10'N, 57°26'W) areas in 2004 and 2005 (Figure 2.1). Furthermore, Terra Nova and Gros Morne National Parks, both in proximate area to these respective urban centers, represented natural areas of little to no disturbance to mosquito breeding sites (Figure 2.2).

The St. John's area is located in the Maritime Barrens Ecoregion with extensive barren areas consisting of mainly dwarf shrub heaths, bogs, shallow fens, as well as forest patches dominated by fir, *Abies balsamea* (Linnaeus) Miller, black spruce, *Picea mariana* Miller, and white spruce, *Picea glauca* (Moench) Voss. Fires occur regularly in this region and sheep laurel, *Kalmia angustifolia* Linnaeus, is the dominant species of the dwarf shrub heath that has replaced a large portion of the forest (Damman 1983). This ecoregion is characterized most clearly by the coolness of the summer and the relatively mild winters and high fog frequency with annual precipitation 1200 to 1700mm (Banfield 1983).

The Deer Lake area lies within the heavily forested and topographically varied Western Newfoundland Ecoregion. *Abies balsamea*, the predominate forest tree throughout; peatlands, covering rather extensive areas; the diverse and luxuriant vegetation of the alluvial alder swamps further characterize this ecoregion (Damman 1983). The most favourable growing conditions found on Newfoundland occur in this

region, due to a relatively long vegetative season and a generally warm summer. The warmest valleys on the island occur in this ecoregion and the Long Range Mountains protect it from cold northeasterly winds, resulting in a distinctly longer frost-free season than adjacent regions (Damman 1983), although much more than its eastern counterpart. Annual precipitation is 900 to 1100mm for lowland areas but can be more moderate near sea level with 1000 to 1200mm (Banfield 1983).

To broaden the coverage of the Province, ten Provincial Parks and one Nature Park were sampled for a larger survey area of the island from east to west coast in 2004. In 2005, additional intensive sampling focused on the west coast of the island and included the town of Cormack, approximately 15 kilometres northeast of Deer Lake, and the city of Corner Brook, approximately 44 kilometres southwest of Deer Lake (Figure 2.2). Cormack is primarily a livestock farming town lying within the Central Lowlands continental climate, in which Deer Lake is situated. However, Corner Brook, an urban coastal city, although within the Western Newfoundland Ecoregion, typically does not experience the prolonged high summer temperatures and is windier than the more sheltered valleys and inland locations (Banfield 1983).

2.2 Larval collection

Digital photographs and maps were consulted to identify standing water or potential breeding sites of mosquitoes in urban areas. Larval sampling procedures followed Service (1993b) using a standard 250mL dipper and average abundances (no./dip) were calculated from five dips per pool. Several site characteristics were recorded including dominant plant species, habitat type, and measurements of larval habitat condition such

as pH, conductivity, and temperature. Sampling in 2004 consisted of a general survey of larvae. Twelve larval sites were identified in the St. John's area and ten in Deer Lake during this year. These sites were sampled in the beginning of the larval mosquito season before the presence/emergence of adults (week 19-23 in St. John's, week 24 & 30 in Deer Lake). Larvae were also collected in Terra Nova and Gros Morne National Parks and Notre Dame Provincial Park during this time. Larvae were sampled more regularly in 2005 from weeks 18 to 30 at 24 sites in the St. John's area, 12 in Deer Lake, 11 in Cormack, and eight in Corner Brook. Larvae were identified to species using Wood et al. (1979) and Darsie and Ward (1981).

2.3 Adult collection

Adult mosquitoes were collected using the New Standard miniature light traps, Model 1012, (John W. Hock Company, Gainesville, FL) following Service (1993a). Light traps were baited with approximately 0.7 kilograms of dry ice in an insulated container suspended from a tree1.5 to 2 metres above ground. Traps were sheltered from the wind as much as possible two to three metres within a tree stand. The traps were placed in the field before dusk and retrieved the following morning; light was deployed from dusk until dawn using the traps photoswitch. Samples were frozen until enumeration and identification in the lab, at which point other large insects were removed and a portion of the remaining sample was chosen at random. Female mosquitoes were removed individually until a count of 30 was reached and the entire sample of mosquitoes was then counted. With the use of a dissecting microscope, the 30 females were identified to species using Wood et al. (1979), Darsie and Ward (1981), and Thielman

and Hunter (2004). When species could not be distinguished, they were either classified as a group (e.g. *Ochlerotatus punctor/abserratus*) or to the level of genus (e.g. *Ochlerotatus* spp.). In the urban areas, transects were established to sample from the rural surrounding environment through the urban core. Sampling sites in St. John's increased from seven in 2004 to ten in 2005 (Figure 2.1) and two five-site transects were chosen in Deer Lake (Figure 2.1). Adult collections expanded in 2005 to include the addition of three trapping sites in the agricultural area of Cormack as well as the urban centre of Corner Brook. Relative adult collection schedules for each location in the two year study are shown in Table 2.1.

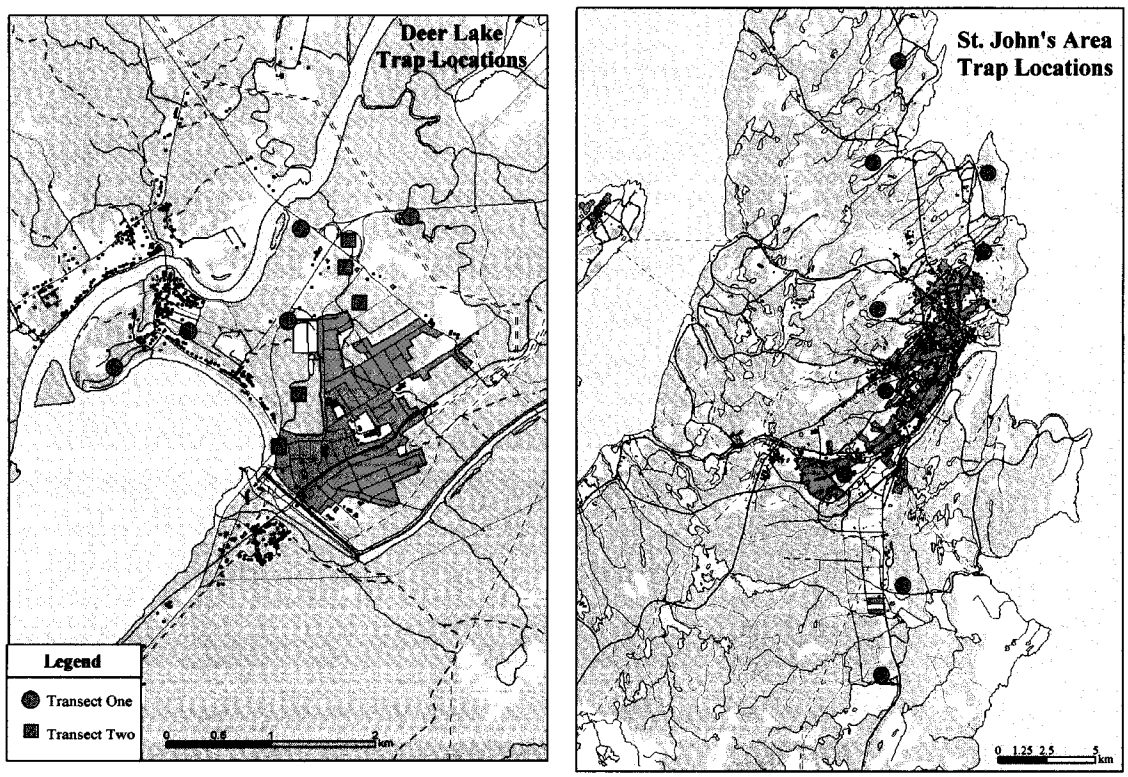


Figure 2.1 Map of adult mosquito trapping sites through Deer Lake and the St. John's area.

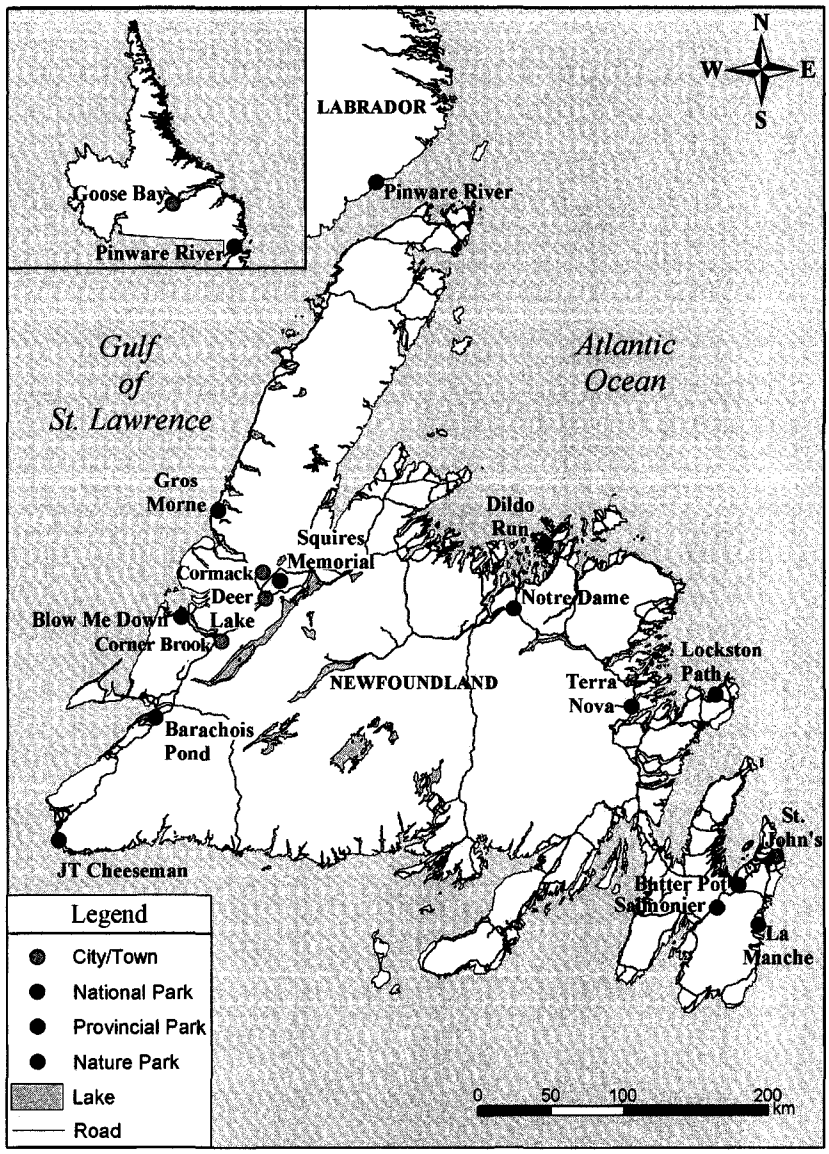


Figure 2.2 Map of Newfoundland and Labrador indicating mosquito sampling locations.

Table 2.1 Adult mosquito trapping schedule indicated by week sampled per year (-).

	2004															
	JUNE				JULY				AUGUST				SEPTEMBER			
	Week															
Location	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
St. John's					-	-	-	-			-	-	-		-	
Deer Lake					-			-	-					-		
Happy Valley-Goose Bay									-			-		-		
Terra Nova National Park																
South West Arm						-										
Newman Sound Campground									-							
Sandy Pond													-			
North West River															-	
Gros Morne National Park																
St. Paul's					-											
Western Brook Pond										-						
Sally's Cove											-					
Green Point														-		
Baker's Brook															-	
Butter Pot Provincial Park					-	-							-			
Notre Dame Provincial Park						-					-					
Squires Memorial Provincial Park							-									
La Manche Provincial Park								-								
Blow Me Down Provincial Park									-							
Lockston Path Provincial Park									-							
Pinware River Provincial Park									-							
Barachois Pond Provincial Park										-						
JT Cheeseman Provincial Park										-						
Dildo Run Provincial Park										-						
Salmonier Nature Park													-			
	2005															
St. John's	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Deer Lake	-	-	-		-	-	-	-		-	-	-	-	-		-
Cormack		-		-		-		-		-		-				
Corner Brook	-		-		-		-				-					
Terra Nova National Park																
South West Arm		-	-	-	-	-	-		-	-			-		-	
Newman Sound Campground		-	-	-	-	-	-		-	-			-		-	
Ken Diamond Park			-	-	-	-	-		-	-			-		-	
Gros Morne National Park																
Rocky Harbour Pond		-	-	-	-	-	-									
Green Point		-	-	-	-	-	-									
Bakers Brook Falls		-	-	-	-	-	-									

3 MOSQUITO FAUNA OF NEWFOUNDLAND

3.1 Introduction

In Chapter 1, it was noted that no thorough study of mosquitoes in Newfoundland had been carried out since the early 1980's, when 24 species were found on the island (Nielsen and Mokry 1982b). The purpose of this study was to re-examine the composition of the mosquito fauna of Newfoundland given the changes in land-use. Thus, one aim of this chapter is to outline species found and their distribution as shown by the current study and compare these findings to previous research. Another objective of the present study was to evaluate the potential for additional species to colonize Newfoundland and the potential of current or new species as vectors of human and animal diseases. These findings can then be used to evaluate the need for further research and monitoring.

3.2 Materials and Methods

Larval and adult mosquito collection and identification followed procedures outlined in Chapter 2. The larval sampling technique used for *Coquillettidia perturbans* followed the methods of Romanowski and Candeletti (1984). DNA extraction and sequencing on the internal transcribed spacer (ITS) gene of 18 individuals, morphologically identified in 2004 as *Culex pipiens/restuans*, was performed using PCR (polymerase chain reaction) amplification by the National Microbiology Laboratory in Winnipeg to confirm morphological identifications. Voucher specimens of all taxa identified have been deposited in the MUN collection.

A species rarefaction (PRIMER v.6β) was calculated using mosquito species abundances collected in each region (St. John's, Eastern Parks, Central Cormack, Deer Lake, Corner Brook, Western Parks, and Labrador). Diversity describes species richness and equitability (or relative abundance of species) and was calculated for mean larval and adult abundances in PRIMER v.6β using the Shannon-Weaver diversity index H′: $H' = -\sum p_i \ln p_i$ where p_i = the proportion of i^{th} mosquito species in the total sample collection for each region.

3.3 Results

A total of twenty-five species of mosquitoes from seven genera was collected in this study; one *Anopheles*, one *Coquillettidia*, two *Culex*, four *Culiseta*, one *Aedes*, 15 *Ochlerotatus*, and one *Wyeomyia* species. A complete species list and distributions of species found in this study are shown in Table 3.1. Five species are reported for the first time in the province, *Ae. vexans*, *Cq. perturbans*, *Cx. pipiens*, *Cs. minnesotae* and *Oc. provocans*. *Aedes vexans* and *Cs. minnesotae* were previously collected (Memorial University of Newfoundland Collection) but not recorded in the literature. Two species are also reported for the first time from the island, *An. earlei* and *Cx. territans*. Nine previously collected species from the province that were not represented in this study include *Cx. restuans*, *Cs. alaskensis*, *Oc. atropalpus*, *Ochlerotatus flavescens* (Muller), *Oc. hexodontus*, *Ochlerotatus impiger* (Walker), *Oc. sticticus*, and *Ochlerotatus stimulans* (Walker).

Table 3.1 Mosquito species recorded from Newfoundland and Labrador with distributions of those species collected during 2004-05.

	St. John's	Eastern Parks	Central Parks	Cormack	Deer Lake	Corner Brook	Western Parks	Labrador
n	456	62	12	128	294	95	47	6
Aedes cinereus Meigen	B	B	B	B	B	L	B	
Aedes vexans (Meigen) [1,3]								
Anopheles earlei Vargas [2]			A		A	L		
Coquillettidia perturbans (Walker) [1]	A	A	A	A	A	A	A	
Culex restuans Theobald [3]								
Culex pipiens Linnaeus [1]				A	B	A		
Culex territans Walker [2]		A		L	L	L		
Culiseta alaskensis Ludlow [3]								
Culiseta impatiens (Walker)		A			B	A	A	A
Culiseta melanura (Coquillett)	A	A						
Culiseta minnesotae Barr	B	A			A	B	A	
Culiseta morsitans (Theobald)	B	B	A	B	B	B	B	A
Ochlerotatus abserratus (Felt and Young)	B	B	A	A	A	A	A	A
Ochlerotatus atropalpus (Coquillett) [3]								
Ochlerotatus canadensis (Theobald)	B	B	B	B	B	B	B	
Ochlerotatus cantator (Coquillett)	B	A	A				A	
Ochlerotatus communis (DeGeer)	A	B	B	B	B	B	B	
Ochlerotatus decticus Howard, Dyar & Knab	L	B			A			
Ochlerotatus diantaeus Howard, Dyar & Knab	A	A			B		A	
Ochlerotatus excrucians (Walker)	B	A	A	B	B	B	A	A
Ochlerotatus fitchii (Felt and Young)				B	B	L	A	
Ochlerotatus flavescens (Mueller) [3]								
Ochlerotatus hexodontus Dyar [3]								
Ochlerotatus impiger (Walker) [3]								
Ochlerotatus implicatus Vockeroth			A	L	B			
Ochlerotatus intrudens Dyar		A			B			
Ochlerotatus nigripes (Zetterstedt)								A
Ochlerotatus pionips Dyar				L	L	L		A
Ochlerotatus provocans (Walker) [1]				B	B	L		
Ochlerotatus pullatus (Coquillett)				A	A			
Ochlerotatus punctor (Kirby)	B	B	B	B	B	B	B	A
Ochlerotatus sticticus (Meigen) [3]								
Ochlerotatus stimulans (Walker) [3]								
Wyeomyia smithii (Coquillett)	L	L						
Number of collected taxa	14	17	11	15	21	16	13	7

[1] New record for the province
[2] New record for insular Newfoundland
[3] Previously collected species not found in this study

A indicates only adult collection
L indicates only larval collection
B indicates larval and adult collections

3.3.1 Notes on new records

Anopheles earlei Vargas

A single larva of this species was collected in Corner Brook, on the outer fringe of the city in a fen among emergent vegetation. Although this is a typical habitat for this species, return visits failed to recover more larvae. One adult was collected in a light trap in Notre Dame Provincial Park, in the north central region of the island, and three in Deer Lake.

Coquillettidia perturbans (Walker)

No larvae of this species were collected although there were repeated attempts to collect larvae by inspecting macrophytes extracted from a known location of abundant adults. Adults were found to have an island-wide distribution. This species was the second most abundant species collected, occurring in very high densities in close proximity to cattail marshes by Lundrigan's Marsh in St. John's and Corner Brook Marsh.

Culex pipiens Linnaeus

Three larvae of this species were collected in Deer Lake. The larvae were found in a ditch and a water filled depression within the town and in association with *Cx. territans*. Adults were collected primarily from Deer Lake, being found in all ten sites within the town, with few collections from Cormack and Corner Brook, two and three individuals respectively.

Culex territans Walker

Larvae were collected in Deer Lake, Corner Brook, and Cormack, primarily in ditches as well as marsh/swamp habitat, agricultural depressions, and stream margins. They were

abundant and found most commonly in association with *Cs. morsitans* and *Cx. pipiens*, as well as *Ae. cinereus*, *Cs. minnesotae*, and *Oc. canadensis*. One adult was collected at Southwest Arm in Terra Nova National Park within fen habitat formed by old beaver dams along a stream surrounded by a coniferous forest.

Culiseta minnesotae Barr

Larvae of this species were collected only once from Corner Brook Marsh, in association with *Cs. morsitans* and *Cx. territans*, and several times from exposed urban and agricultural ground depressions filled with water in St. John's, in association with *Ae. cinereus*. Larvae were collected in dense clumps of vegetation, predominately sedge. Light trapped adults were collected island-wide throughout the season.

Ochlerotatus provocans Walker

Larvae were collected primarily in Cormack ditches and woodland snowmelt pools dominated by sedges. One and two larvae of this species were collected in Corner Brook and Deer Lake respectively. The larvae were most commonly found in association with *Ochlerotatus communis* (DeGeer), and *Ochlerotatus abserratus* (Felt and Young), as well as with *Ae. cinereus*, *Ochlerotatus intrudens* Dyar, *Ochlerotatus fitchii* (Felt and Young), and *Oc. canadensis*. Adults were collected in conifer forested locations, primarily in low abundance in Deer Lake and two individuals were found in Cormack.

3.3.2 Overview of mosquito survey

Frequency of occurrence and abundance results of larval and adult surveys are shown in Appendix 1. Four species, in order of abundance, *Oc. canadensis*, *Oc. communis*, *Ochlerotatus punctor* (Kirby), and *Cx. territans*, accounted for over 84% of

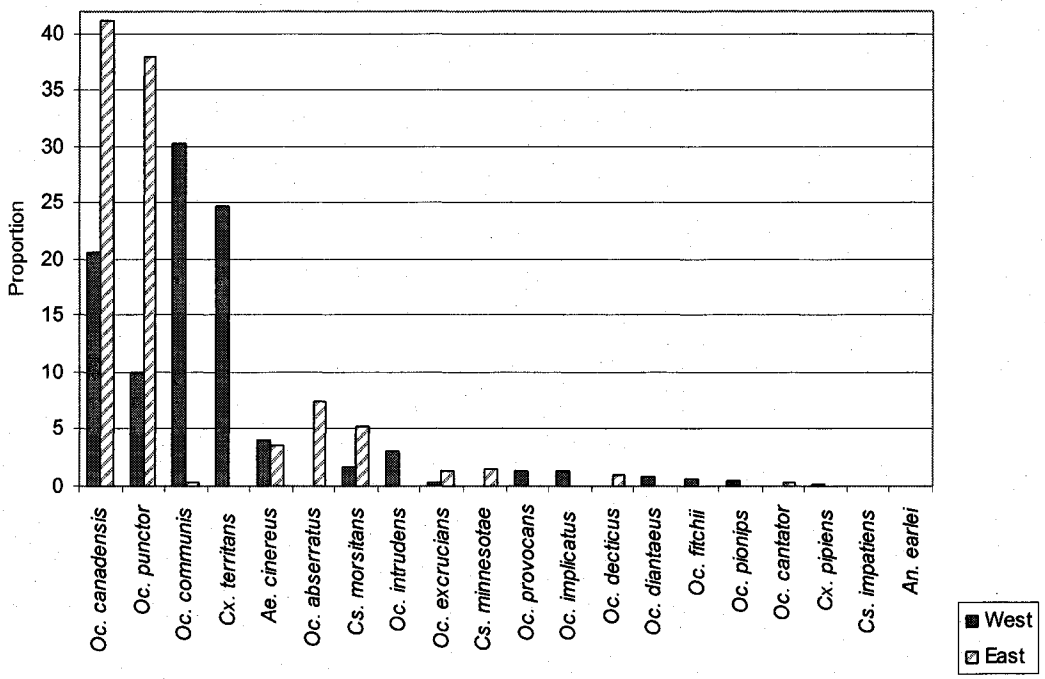
the total 3167 larvae collected. Six species of adults, in order of abundance, *Oc. canadensis*, *Cq. perturbans*, *Oc. abserratus/punctor*, *Oc. communis*, *Cs. morsitans*, and *Ochlerotatus excrucians* (Walker), accounted for almost 82% of the total 5276 identified from subsamples of the 46,006 collected. Figure 3.1 shows proportional abundances of mosquito species collected in the western and eastern regions of insular Newfoundland. Of the 25 species collected, 88 percent, or 22 species were represented in the western portion of the island, compared to only 68 percent in the eastern portion with 17 species collected. *Culex pipiens*, *Oc. fitchii*, *Oc. pionips*, *Ochlerotatus provocans* (Walker), and *Ochlerotatus pullatus* (Coquillett) were found only in the west. *Culiseta melanura* and *Wy. smithii* were collected only in the east.

Results of the adult and larval diversity analysis are shown in Table 3.2. The richest fauna occurred at Deer Lake in which 15 and 18 mosquito species were captured of the total 20 larval and 23 adult mosquito species found. However, the region with the highest Shannon-Weaver diversity index value for larvae (1.78) was Cormack with 11 species. Five species, Oc. *canadensis* (33.6%), *Oc. communis* (29.5%), *Oc. punctor* (12.4%), *Ae. cinereus* (8.5%), and *Cx. territans* (6.4%) accounted for 90.4% of larvae identified from this region. In order to make direct comparisons of species richness among locations where different numbers of larvae were found, a rarefaction calculation for randomly collected larvae from each location was performed. Cormack had the highest species richness of 8.4 species (Figure 3.2). Deer Lake had the highest diversity index value (2.38) for adult mosquitoes and six species, *Cq. perturbans* (19.4%), *Oc. excrucians* (12.7%), *Oc. diantaeus* (11.9%), *Oc. canadensis* (10.6%), *Oc. communis*

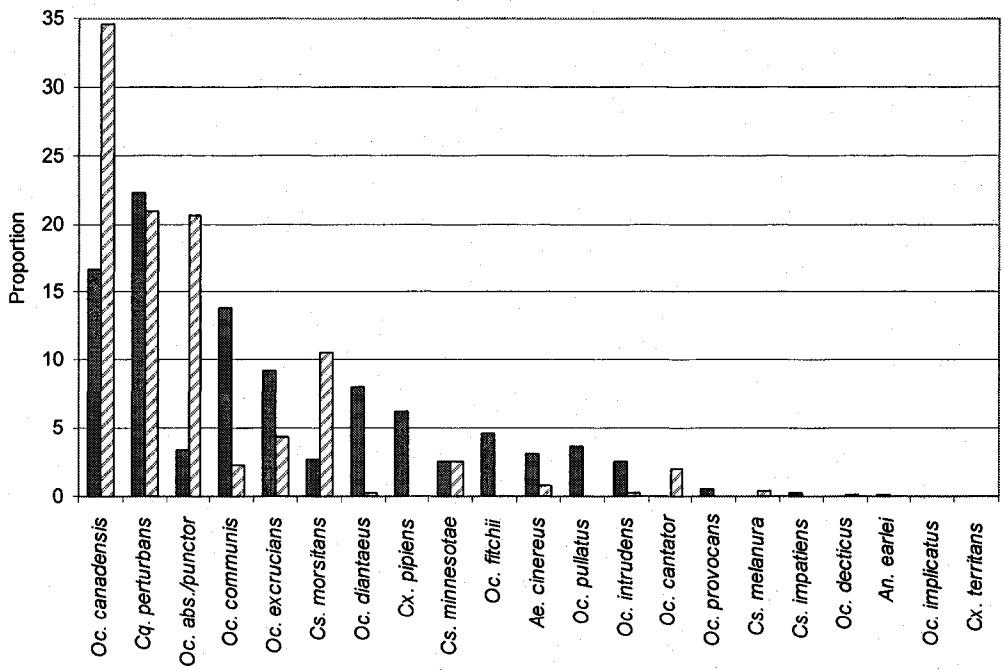
(10.3%), and *Cx. pipiens* (9.1%), accounted for 73.9% of adults identified from this region. To be able to compare species richness among areas where different numbers of adults were collected, rarefaction calculations were made on the samples of 30 random individuals identified from each location. Deer Lake had the highest species richness of ten species (Figure 3.3).

Table 3.2 Comparison of community structure of adult and larval mosquitoes of Newfoundland and Labrador, 2004-05.

Region	No. of species		Larval Diversity Index H'	Adult Diversity Index H'
	Larvae	Adults		
Labrador	0	6	0.00	1.48
Western Parks	4	12	1.13	1.37
Corner Brook	12	8	1.57	1.15
Deer Lake	15	18	1.67	2.38
Cormack	11	11	1.78	1.51
Central Parks	4	10	1.36	1.74
Eastern Parks	7	15	1.54	1.79
St. John's	9	10	1.39	1.62



a)



b)

Figure 3.1 Proportional abundances of larvae a) and light trapped adult b) mosquito species collected regionally across insular Newfoundland, 2004-05.

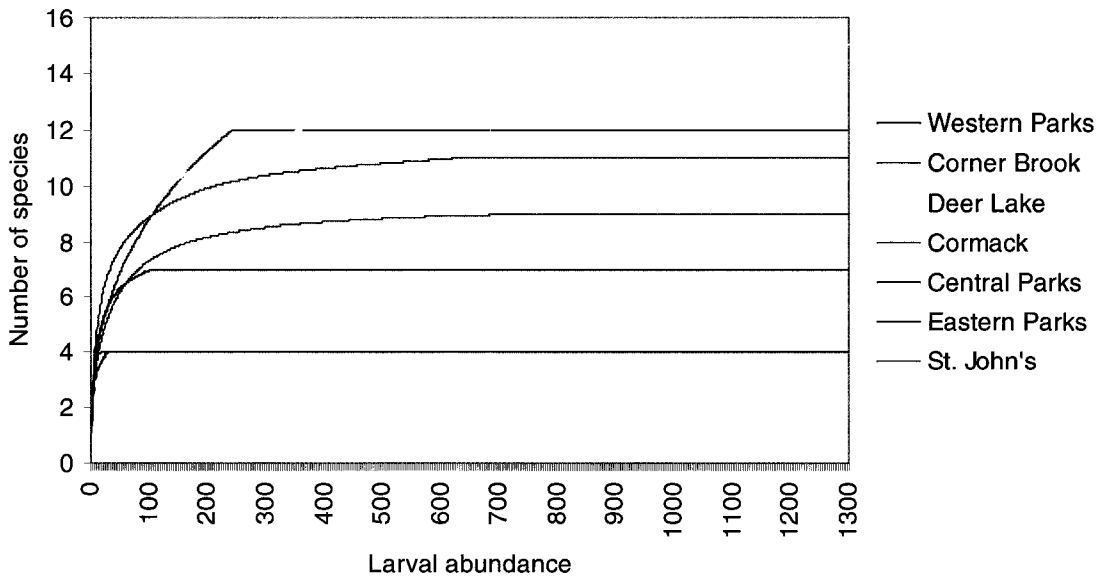


Figure 3.2 Rarefaction curves for mosquito larval communities in Newfoundland and Labrador calculated from total number of larvae collected.

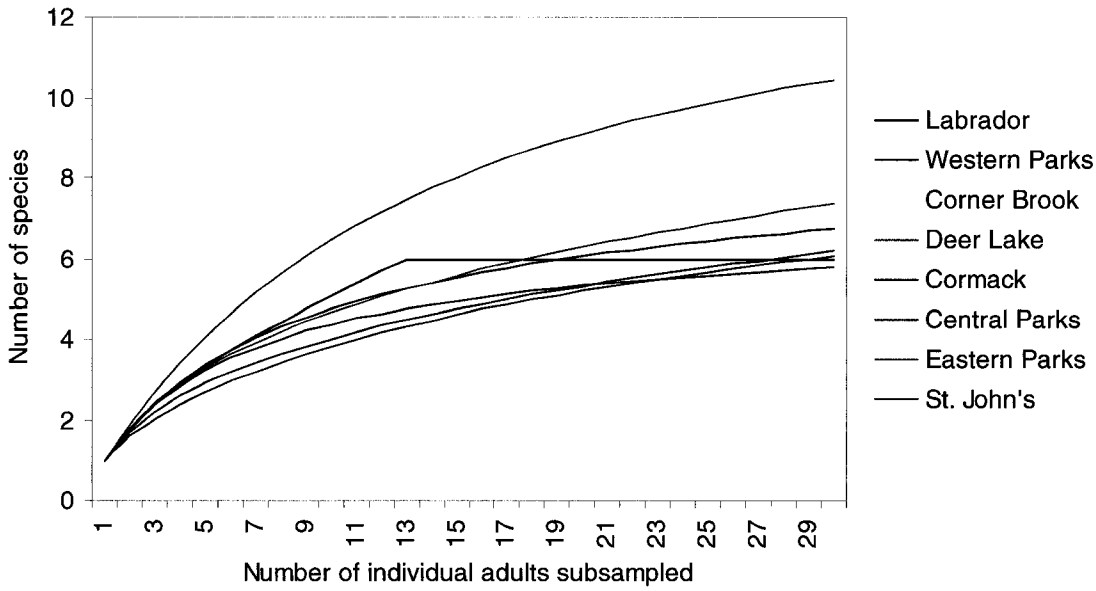


Figure 3.3 Rarefaction curves for adult mosquito communities in Newfoundland and Labrador.

3.4 Discussion

The current study recorded five species new to the island and thus shows the value of monitoring the mosquito fauna over time. Mosquito species not detected by repeated surveys are either ones that are rare and thus not often taken in sampling programs (Wood et al. 1979), occur in special habitats, e.g. *Wy. smithii* in pitcher plants, or are species extending their range from elsewhere (Nielsen and Mokry 1982a). Thus even in well surveyed areas additional taxa are being found (Darsie and Ward 2000).

The new record of *Cx. pipiens* for the province is of particular interest epidemiologically because of its known importance as an effective vector in the transmission of SLE and WNV in parts of central and eastern North America (Drebot et al. 2003, Wood et al. 1979, Turell et al. 2002). The importance of this species and similar *Culex* species, such as *Cx. restuans*, is primarily due to two main aspects of their biology. First, they are almost exclusively bird biting mosquitoes (Turell et al. 2002) and as such, are responsible for driving the amplification cycle in the avian population. Secondly, they are prolific breeders preferring to lay their eggs in stagnant enriched water, a habitat which is often increased by human land-use practices. Therefore, either *Cx. pipiens* or implicated bridge vectors have the opportunity to infect mammals of close proximity to breeding sites.

The majority of positive mosquito pools for WNV in Canada were comprised of *Cx. pipiens* and have been found in Ontario, Manitoba, and Quebec (Drebot et al. 2003), and its range in Canada extends from Ontario to Nova Scotia and Prince Edward Island (Wood et al. 1979). The presence of adults and larvae of this species in Deer Lake

indicates an established population in this area. The proportion of *Cx. pipiens* in relation to other species is low in the current study, however dry-ice baited miniature light traps are not effective for population sampling of this species and are more likely to collect certain *Aedes* or *Ochlerotatus* species (Turell et al. 2002, Allan and Kline 2004). Therefore, *Cx. pipiens* complex females are likely under-represented in Newfoundland collections. Furthermore, *Cx. pipiens* is a tree-canopy species (Anderson et al. 2004) that tends to feed primarily on birds in more northern latitudes (Spielman 2001), so at trap height, 1.5 to 2 meters above the ground, population abundances may be further underestimated. Hence, low numbers of *Cx. pipiens* in the current study may not necessarily indicate low numbers in the environment.

In addition, few larvae of *Cx. pipiens* were collected in Deer Lake. Larger numbers of mosquito predators such as Dytiscidae (diving beetles), Chaoboridae (phantom midge), Odonata (dragonflies), and Hemiptera, such as backswimmers, were observed in ditches in the Deer Lake area compared to similar habitat elsewhere on the island, although this was not quantified. Although all of these groups of insect predators feed on mosquitoes, the Coleoptera (beetles) are considered the most efficient and can reduce larval mosquito populations (Woodring and Davidson 1996). Further investigation is warranted in identifying *Cx. pipiens* breeding locations in the Deer Lake area. This species is decidedly urban and reaches greatest numbers in large urban centers, generally associated with water that has a high organic content and can be found in a fairly wide range of container habitats (Kronenwetter-Koepel et al. 2005, Beehler and Mulla 1995). Catch basins and storm drains provide ideal habitat for *Cx. pipiens* and also offer the advantage

of evading predators that would otherwise occur in natural waterways. A closer look at such habitats could reveal *Cx. pipiens* larvae in Deer Lake. In addition, the limited number of individuals of this species collected could be attributed to such container breeding behaviour and field collections could have missed developing larvae in such small or restricted environments, especially since this species can develop from egg to adult in as few as eight days (Wood et al. 1979).

Coquillettidia perturbans is distributed throughout North America and can be a serious pest of humans and livestock in parts of southern Canada adjacent to large permanent marshes (Wood et al. 1979). High abundances of this species in such habitat dominated by cattails can now be seen across the Island of Newfoundland. Eastern Equine Encephalitis virus has been isolated from *Cq. perturbans* which acts as a bridge vector of this disease (Wood et al. 1979, Romanowski and Candeletti 1984, Bosak et al. 2001). In Canada, Western Equine Encephalitis virus was also isolated from this species although the primary enzootic vectors of EEE and WEE are *Cs. melanura* and *Culex tarsalis* Coquillett respectively (Wood et al. 1979, Artsob 1990).

It was surprising that no complaints about the adult activity of *Cq. perturbans* were voiced by residents near Lundrigan's Marsh, St. John's and Corner Brook Marsh given its indiscriminate feeding behaviour and the thousands of adults trapped within the two cities. This curiosity raises the question of what hosts were being used for blood meals. Historically, *Cq. perturbans* larvae are hard to sample because they remain buried in the mud attached to the roots of emergent vegetation, where they remain throughout development. Attempted larval collections from Lundrigan's Marsh in St. John's failed

to turn up any individuals. *Coquillettidia perturbans* larvae are very quick to remove themselves from the host plant when disturbed (Romanowski and Candeletti 1984) and larval populations are clumped (Batzer 1993), therefore in selecting sampling sites from the large cattail marsh they may have been missed

The distribution of *Cs. melanura* on the island appears to be restricted to the eastern region with few adults collected in St. John's and Terra Nova National Park. These findings support those of Nielsen and Mokry (1982a) who reported collecting a larva in this area, and later adults were also trapped near St. John's. *Culiseta melanura*, the primary enzootic mosquito vector of EEE (Wood et al. 1979, Morris et al. 1980, Garvin et al. 2004), is common in the eastern US, although its only collections in Canada were from southern Quebec and Ontario (Wood et al. 1979, Darsie and Ward 2000) until its appearance in Newfoundland (Nielsen and Mokry 1982a). This collection represented a considerable northern extension of this species range; however its rare occurrence and low abundance in Canada make it an unlikely vector of EEE and human pest.

The current study shows a range extension on the island for *Oc. pionips* and *Oc. pullatus*. Formerly collected only on the northern peninsula (Nielsen and Mokry 1982b), these two species now occur in Cormack and Deer Lake, and *Oc. pionips* was also collected in Corner Brook. In addition, although active collections of *Wy. smithii* were only conducted in the eastern region of the province, its distribution is known to be island-wide and into Labrador, following the distribution of the pitcher plant, *Sarracenia purpurea* Linnaeus (Wood et al. 1979).

There were no records of *Cs. minnesotae* and *Oc. provocans* in Newfoundland prior to this study. Wood et al. (1979) recognized *Cs. minnesotae* as the least known Canadian mosquito, with larvae first being collected in Canada in the early 70's from a permanent cattail marsh in Manitoba. Collections have appeared as far east as Quebec. However, this species seems to be distributed island-wide and first appeared as overwintering females when light traps were deployed in early June. Adults were collected until the end of August, with few larvae collected in July. Distributed from British Columbia to Nova Scotia, *Oc. provocans* is one of the first species to emerge in the spring and is seldom seen after the other snowmelt species have emerged (Wood et al. 1979). Few individuals of this species were collected in the west with larvae first appearing in early May and only one adult after the end of June, which concurs with the literature.

Anopheles earlei and *Cx. territans* were previously recorded from Labrador, although they were not collected on the island prior to this study. *Anopheles earlei* is common across most of Canada, and generally appears as an overwintering adult early in the spring with fewer adults present in the summer (Wood et al. 1979). However, our limited collections occurred in July and August. The presence *of Cx. territans* in Labrador is not unexpected as it is primarily an amphibian feeder and six species of amphibians are native to this region including three frogs, one toad, and two salamanders (Campbell et al. 2004). Insular Newfoundland lacks native amphibians, however four species have been successfully introduced. Green frogs are now distributed across the island after their introduction to St. John's about 1850, the American toad and Wood frog thrived after their introduction to Corner Brook in the 1960's, and the recently discovered

Mink frog was found near Corner Brook (Campbell et al. 2004). The thriving population of *Cx. territans* indicates that they have followed these successful amphibian introductions. The high abundance of *Cx. territans* larvae in Deer Lake, Cormack, and Corner Brook correlates with the greater diversity of these amphibians on the west coast of the island. In addition, within or adjacent to breeding sites of *Cx. territans* in Deer Lake, large numbers of tadpoles and frogs were observed.

Southwest Arm in Terra Nova National Park, where an adult *Cx. territans* was collected, is located in the proximate area of a characteristic fen habitat created by an old beaver dam where large numbers of frogs are present. Small numbers of adult mosquitoes in trapping locations adjacent to *Cx. territans* breeding habitat is possibly the result of trapping technique. This species' specialized feeding preference would be a factor if the rate of dry ice sublimating was at a concentration not attractive to *Cx. territans*, as different types of animals release different amounts of carbon dioxide (Service 1993a).

Collections in Labrador were limited and included only three samples from Happy Valley-Goose Bay and Pinware River Provincial Park, thus only six of the twenty-seven species previously recorded from Labrador were found. Many northern species, including *Cs. alaskensis*, *Oc. flavescens*, *Oc. hexodontus*, and *Oc. impiger* were not recorded in the current study. The only species collected from Labrador and not on the island was *Ochlerotatus nigripes* (Zetterstedt). Of the twenty-seven mosquito species recorded in Labrador through previous research, six are exclusive to this region of the province. There are obvious ecological influences on the mosquito composition of the

province given that species are represented in Labrador and not on the island, and vice versa. Furthermore, the potential for colonization of species in Labrador as part of mainland North America may be greater if the distance over open water to the island is limiting. However, if opportunity existed for transport to the island from Labrador by human activity or weather fronts, it is possible that additional species will establish breeding populations, as seen with *An. earlei* and *Cx. territans*.

The first record of *Ae. vexans* comes from a collection made on July 13, 1989 by M. Colbo in Goose Bay, Labrador, in which one adult specimen appears in the MUN Culicidae collection. In addition, there is an unpublished report by J. Mokry of *Cx. restuans* from the island and an additional *Cx. pipiens/restuans* specimen is also present in the collection from Newfoundland, collected by J. Phipps. It is not unusual for Newfoundland to receive insects via air currents. Morris (1983) named this method the 'drift migrant method' and pointed out several species which occur in Newfoundland every year, although they do not overwinter here, including the monarch butterfly, *Danaus plexippus* (Linnaeus), and the corn ear worm, *Heliothis zea* (Boddie). Another example is of the diamondback moth, *Plutella xylostella* Linnaeus, which is not native to Newfoundland and Labrador, but arrives each summer on wind currents from overwintering sites in the United States (Hermanutz et al. 2004).

Wind-drifting taxa are often small organisms, such as dipterans, which can be physically swept upwards and transported by storms and other large-scale atmospheric phenomena. In addition, drifting with ambient winds is a mode of transport available to many insects. Wolf et al. (1986) found through radar observations and collections of

insects in the Gulf of Mexico, that longer flight time when traveling over water enables insects to cross wide bodies of water, especially when the winds are strong. Various collections of Culicidae showed the presence of some species of mosquitoes 32 to 200 kilometres from the nearest mainland (Wolf et al. 1986).

It can be speculated that many mosquito species arrived in Newfoundland by wind-mediated displacement, although some did not establish a breeding population and hence few specimens have been collected. These include *Cx. restuans*, *Ae. vexans*, *Oc. sticticus*, and *Oc. stimulans*. Furthermore, the dispersal capability of mosquitoes assisted by human travel is well documented in the literature. It has been found that mosquitoes can establish after the transport of adults in airplanes and boats, as well as larvae in ship ballast water and tires (Calder and Laird 1994, Lounibos 2002). For those species which have been successful arriving and establishing a population on the island, such as *Cx. pipiens*, *Oc. provocans*, and *Cq. perturbans*, suitable conditions must have existed and the distance to the mainland must not be a limiting factor.

It was shown by the Shannon-Weaver Diversity Index (H′) that the western portion of the province is more diverse in mosquitoes than its eastern counterpart. This difference may be because not enough habitat types were represented on either side to obtain a representative sample population, in which case the diversity index may not be accurate. For example, we know that *Wy. smithii* occurs in the west (Heard 1994), but this species was not recorded in this region during the present study since pitcher plants there were not sampled from. In addition, from the species rarefaction curves, it was clear that this calculation cannot be computed for certain locations because of insufficient

mosquito abundance data due to limited sampling or trapping effort. A difference in sampling effort for each region may also affect the accuracy of the diversity index values. Differences in species diversity from west to east on the island may be attributable to winds coming from the mainland. However, a more probable explanation is the many environmental conditions which could influence the establishment of populations in each region, and as such be reflected in the diversity of the area. These conditions can include local climate and habitat differences, as well as anthropogenically enhanced breeding sites for mosquitoes, which will be investigated in the subsequent chapter.

4 THE IMPACT OF LAND-USE ON MOSQUITO POPULATIONS OF NEWFOUNDLAND

4.1 Introduction

In order to evaluate the impact of land-use, baseline mosquito studies are needed to form a database for comparison. To acquire these data, mosquito studies have examined breeding habitats in areas where no long-term information on species habitat utilization and population dynamics exist (Dahl et al. 2004). These studies are needed in urban, and particularly rural areas, where these baseline data often do not exist (Kronenwetter-Koepel et al. 2005), and in areas where the knowledge of mosquito biology is incomplete (Rydzanicz and Lonc 2003). This research is often initiated after changes in land-use (Hearnden and Kay 1995), catastrophic flooding events (Rydzanicz and Lonc 2003), and risk of pathogen transmission by mosquito vectors are recognized. In addition to impacting larval populations directly, these changes may also affect adult mosquitoes. Mating and resting sites may be disrupted and potential changes in the availability of blood-host populations, such as increases in domestic animals and avian species as well as wild host populations may result. The risk of disease transmission is an issue in populated areas, especially when land-use changes and flooding events have the potential to change both the occurrence of mosquito species and/or their abundance. For this reason vectors are often the focus of these studies which evaluate productivity of habitat types (Kronenwetter-Koepel et al. 2005) and the effects of nutrient load on their growth (Reiskind et al. 2004) and oviposition behaviour (Reiskind and Wilson 2004). Data from

such studies determine the risk of disease to humans and animals and are often used to construct integrated mosquito control strategies.

The objective of the present investigation was to evaluate the impact of land-use on mosquito occurrence and populations. This investigation was particularly focused on the influence of urbanization and agricultural activities on mosquito breeding, population composition, and abundance in various habitats across these landscapes.

4.2 Materials and Methods

Larval and adult mosquito collections and identification followed procedures outlined in Chapter 2. Larval habitat was further classified by land-use into natural and disturbed sites. Disturbed sites were those within or near an urban centre or agricultural development which could be influenced by land alterations and/or increased nutrient load. Classification of habitat types included bog, ditch, fen, marsh/swamp, temporary pool, pond margin, and stream margin. Habitat type classified as temporary pool represented ground pools or depressions which fill with water by snowmelt or precipitation events. Stream/pond margins were the peripheral habitat of water bodies which are influenced by floodwater. Environment Canada temperature data were used and daily mean temperatures to calculate a weekly mean temperature prior to trapping in a particular location.

Data were entered into Microsoft Excel and all statistical tests were conducted with SAS version 9.1. Presence/absence data were analyzed using a Logistic Regression and logit link function to evaluate the frequency of occurrence of mosquito species among regions, habitats, or sites. To explain variation in the abundance of mosquito species,

these predictor variables were tested using a Generalized Linear Model with a negative binomial distribution and log link. This distribution was used for count data because of the non-normal distribution of residuals, however, if it was not appropriate due to homogeneity of the residual deviance, then the Poisson distribution with a Pearson scale transformation was used. Mean abundance data of specific adult species were analyzed using a Logistic Regression and a cumulative logit link function to test the difference of seasonal abundance between regions as related to temperature. Analysis of larval and adult abundance data used only instances where they were present. A type 3 analysis was computed when categorical predictor variables were used and all statistical tests were considered significant at $p< 0.05$.

4.3 Results

4.3.1 Mosquito larvae occurrence and abundance

4.3.1.1 Regional analysis of mosquito larvae populations

Twenty-one species of larval mosquitoes were collected during the study and their distributions are shown in Table 3.1 (Chapter 3). The frequency of occurrence for all mosquito larvae identified was not significantly different ($p= 0.3929$) between eastern and western regions of the Island of Newfoundland. Larvae occurred 49 percent of the time in the east compared to 39 percent in the west (Table 4.1). A significant difference was found in the mean abundance ($p= 0.0160$) of mosquito larvae between these regions (Table 4.1); higher mean abundances were found in the west. Among the 21 species collected, the abundances of seven species each accounted for over five percent of the

total collected, however, *Cx. territans* was collected only in the west, and *Oc. abserratus* was collected only in the east, as shown in Table 4.1. Of those seven species, no significant differences in the occurrence of *Ae. cinereus* and *Cs. morsitans* were found between regions. However, the frequency of occurrence of *Oc. canadensis*, and *Oc. punctor* was significantly higher in the east compared to the west, p= 0.0001 and 0.0005, respectively (Table 4.1). *Ochlerotatus communis* occurred significantly more frequently (p=0.0141) in the west compared to the east. The mean abundances of these seven species combined were not significantly different between regions. Although not significant, *Ae. cinereus*, *Oc. canadensis*, and *Oc. communis* were found in higher mean abundances in the west and *Oc. punctor* in the east (Table 4.1).

Table 4.1 Cumulative frequency of occurrence and mean abundance (measured as numbers per dip) of seven most abundant species of mosquito larvae and total larvae identified between Eastern and Western regions of the Island of Newfoundland, 2004-2005 (SD= standard deviation).

	Cumulative frequency of occurrence			**Mean abundance (SD)**		
	East	West		East	West	
n	270	404	p-value	133	159	p-value
Ae. cinereus	12	20	0.7622	0.5 (0.2)	0.8 (1.7)	0.4987
Cs. morsitans	20	17	0.0776	0.4 (0.2)	0.4 (0.3)	0.7846
Cx. territans	0	60	n/a	0.0 (0.0)	1.6 (1.3)	n/a
Oc. abserratus	14	0	n/a	0.8 (1.0)	0.0 (0.0)	n/a
Oc. canadensis	70	57	0.0001	0.9 (0.9)	1.4 (2.8)	0.1386
Oc. communis	1	18	0.0141	0.5 (0.0)	6.6 (14.2)	0.5910
Oc. punctor	55	43	0.0005	1.1 (0.9)	0.9 (1.3)	0.5476
Total species	133	159	0.3929	0.8 (0.9)	1.5 (4.3)	0.0160

Note: n/a refers to analysis not being applicable due to complete absence of species in a region

4.3.1.2 Habitat type analysis of mosquito larvae populations

The presence and mean abundance of all larvae identified within different habitat types were examined between eastern and western regions of the island. Table 4.2 shows larvae occurred most often in habitats classified as temporary pools, fens, and ditches in the east and in ditches, temporary pools, and marshes/swamps in the west. When these regions are compared, mosquito larvae were found more often in bogs, fens, and temporary pools in the east, and in ditches and stream/pond margins in the west. Furthermore, larvae were found in marsh/swamp habitat only in the western region. The frequency of occurrence of larvae in these habitat types between regions was not significantly different (Table 4.2).

There were significant differences in the mean abundance of larvae in bogs ($p= 0.0016$) and temporary pools ($p= 0.0100$) habitat between regions. A higher mean abundance of larvae was found in bogs in the east compared to the west. Conversely, a higher mean abundance was found in habitat types classified as temporary pools in the west compared to the east (Table 4.2). Table 4.3 shows that only three of the seven most abundant species on the Island of Newfoundland were found in all the habitat types. There was a significant difference in the frequency of occurrence of two of the three species, *Oc. canadensis* ($p= 0.0069$) being found most often in temporary pools and ditches, and *Cx. territans* ($p= <0.001$) being found most often in ditches and marsh/swamp habitats (Figure 4.1). The mean abundance of these two species was also significantly different between habitat types with *Oc. canadensis* ($p= 0.0013$) and *Cx. territans* ($p= 0.0002$) in highest mean abundance in temporary pool and stream/pond margins respectively (Table 4.3 & Figure 4.2). Table 4.3 shows no significant

differences in the mean abundance of other species and total identified mosquito larvae between habitat types.

Table 4.2 Cumulative frequency of occurrence and mean abundance (measured as numbers per dip) of total mosquito larvae identified in habitat types in Eastern and Western regions of the Island of Newfoundland, 2004-2005 (SD= standard deviation).

	Cumulative frequency of occurrence			Mean abundance (SD)		
	East	West		East	West	
n	270	404	p-value	133	159	p-value
Bog	6	7	0.9590	2.0 (1.9)	0.3 (0.2)	0.0016
Fen	45	7	0.4624	0.8 (0.7)	0.5 (0.4)	0.3398
Marsh/Swamp	0	39	n/a	0.0 (0.0)	1.4 (1.5)	n/a
Ditch	32	99	0.5286	0.8 (0.9)	1.1(1.6)	0.3198
Temporary pools	92	86	0.0566	0.7 (0.8)	2.3 (7.1)	0.0100
Stream/pond margin	14	23	0.6029	0.9 (0.7)	1.0 (1.3)	0.7970

Note: n/a refers to analysis not being applicable due to absence of individuals in a habitat type

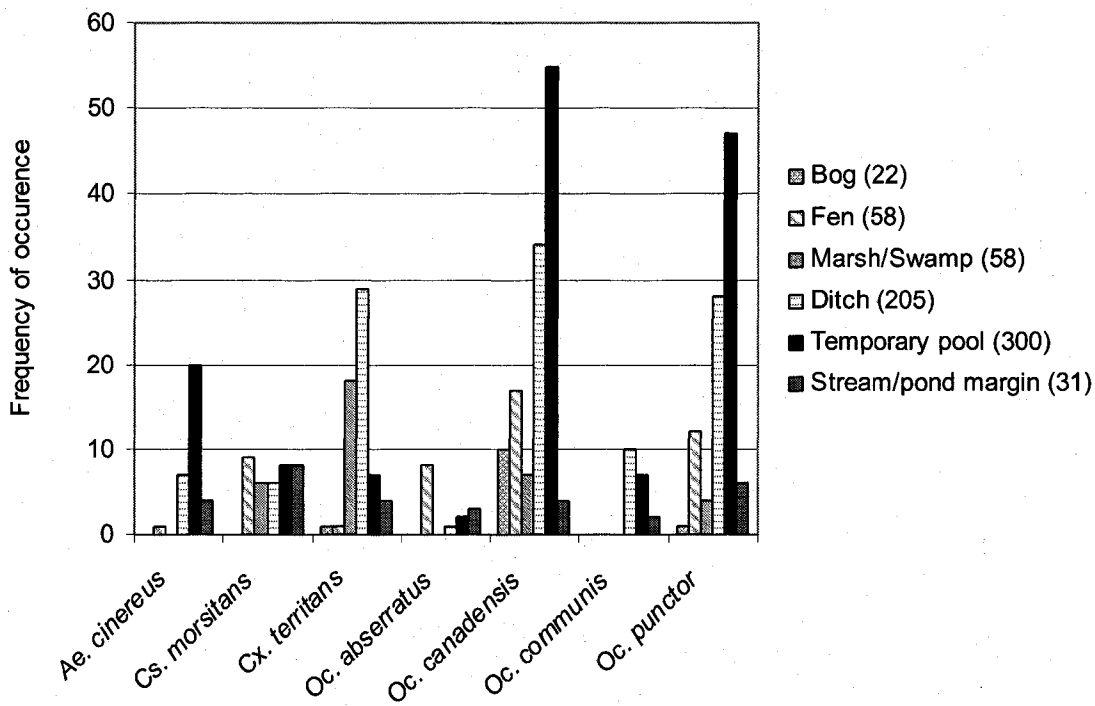


Figure 4.1 Cumulative frequency of occurrence of the seven most abundant species of mosquito larvae identified in habitat types (n) on the Island of Newfoundland, 2004-2005.

Table 4.3 Mean abundances (measured as individuals per dip) of the seven most abundant species of mosquito larvae and total larvae identified in habitat types on the Island of Newfoundland, 2004-2005 (SD= standard deviation).

	Bog	Fen	Marsh/ Swamp	Ditch	Temporary pools	Stream/ pond margin	p-value
n	11	36	28	85	114	18	
Ae. cinereus	0.0 (0.0)	0.4 (0.0)	0.0 (0.0)	1.4 (2.8)	0.5 (0.4)	0.3 (0.1)	0.1926
Cs. morsitans	0.0 (0.0)	0.4 (0.3)	0.5 (0.3)	0.4 (0.2)	0.3 (0.2)	0.4 (0.2)	0.7367
Cx. territans	0.2 (0.0)	0.8 (0.0)	2.4 (1.7)	1.1 (0.8)	1.2 (0.7)	2.9 (1.1)	0.0002
Oc. abserratus	0.0 (0.0)	0.9 (1.1)	0.0 (0.0)	0.1(0.0)	0.4 (0.4)	1.1 (1.2)	0.6424
Oc. canadensis	1.3 (1.6)	1.0 (0.8)	0.5 (0.2)	1.0 (1.7)	1.3 (2.6)	0.8 (0.8)	0.0013
Oc. communis	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.4 (0.3)	15.6 (20.4)	2.6 (2.8)	0.8860
Oc. punctor	0.7 (0.0)	0.7 (0.5)	0.9 (0.7)	1.3 (1.7)	0.9 (0.9)	0.8 (0.7)	0.5197
Total species	1.1 (1.5)	1.0 (1.4)	0.7 (0.7)	1.4 (1.5)	1.5 (5.0)	1.0 (1.1)	0.5480

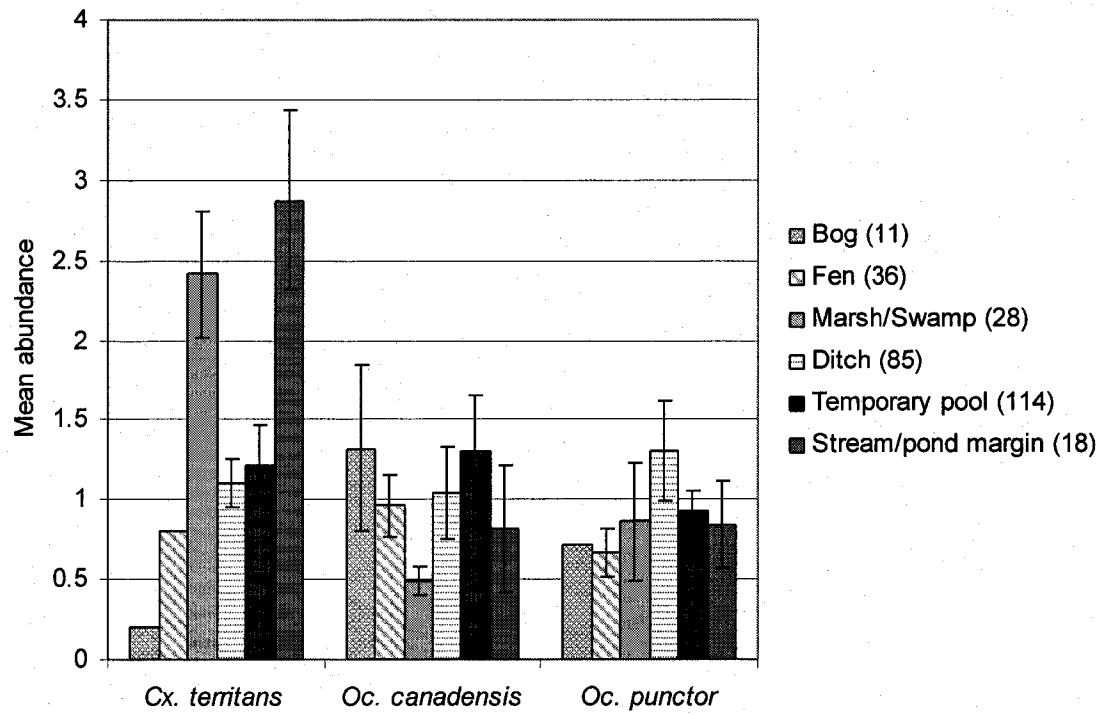


Figure 4.2 Mean abundances (measured as individuals per dip) of three abundant species of mosquito larvae identified in habitat types (n) on the Island of Newfoundland, 2004-2005.

4.3.2 Mosquito larvae of St. John's, Eastern Newfoundland

Among ten species of larvae identified in St. John's, a significant difference in their frequency of occurrence (p= 0.0236) was found between land-use categories. Larvae occurred more frequently in natural and disturbed wetland habitats and least often in agricultural habitats. This result was significant (p= 0.0018) when the three most abundant species, *Oc. abserratus*, *Oc. canadensis*, and *Oc. punctor*, were tested for differences between land-use categories. When these species were analyzed separately, only the frequency of occurrence of *Oc. punctor* was significantly different between these land-uses (p= 0.0255). Figure 4.3 shows all three species occurred most often in natural and disturbed wetlands. In habitats where *Oc. punctor* occurred, it was collected least often from urban habitats, *Oc. abserratus* and *Oc. canadensis* were uncommon in agricultural habitats, and *Oc. abserratus* was not collected in an urban habitat.

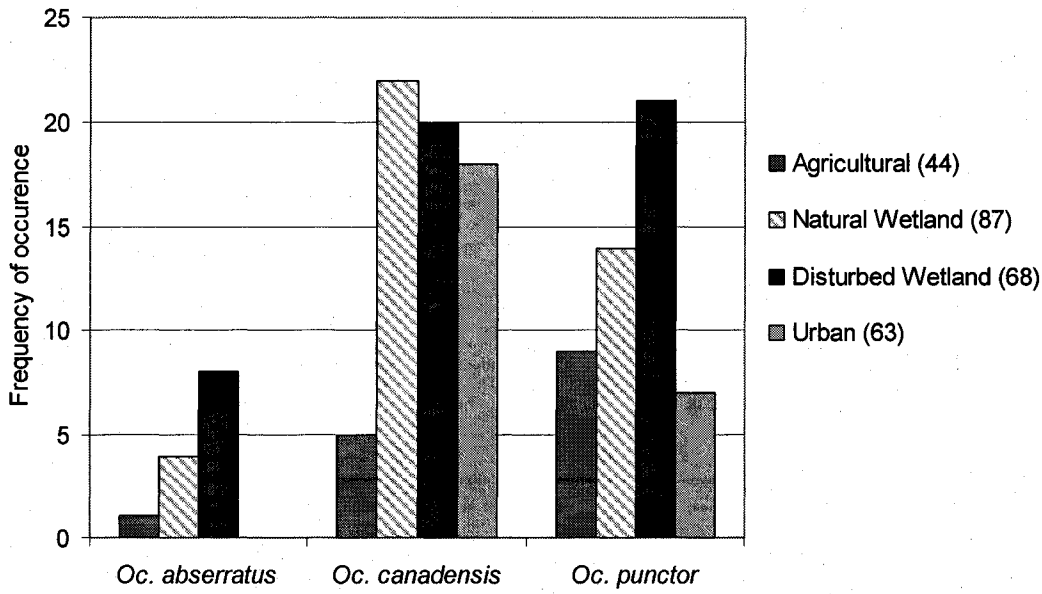


Figure 4.3 Cumulative frequency of occurrence of the three most abundant species of mosquito larvae identified from land-use categories (n) in St. John's, Eastern Newfoundland, 2004-2005.

When the mean abundance of all species of larvae between land-use categories was analyzed, there was a significant interaction ($p= 0.0024$) between land-use and species. However, due to the difficulty in interpreting this result with all species included, the same analysis was performed on the three most abundant species and again a significant interaction ($p= 0.0004$) resulted. Figure 4.4 shows that the mean abundances of these species varied between land-use categories with the highest mean abundance of *Oc. canadensis* occurring in natural wetlands, *Oc. punctor* in agricultural and urban habitats, and *Oc. abserratus* in natural and disturbed wetlands. Significant differences in the mean abundance of the total mosquito larvae and the most abundant species between land-use categories were also found when analyzed without an interaction, $p= 0.0336$ and 0.0470 respectively. Both results showed highest mean abundances in natural wetlands followed by agricultural habitats. When species were analyzed separately, only the mean abundance of *Oc. canadensis* was significantly different between land-use categories ($p= <0.0001$) (Figure 4.4).

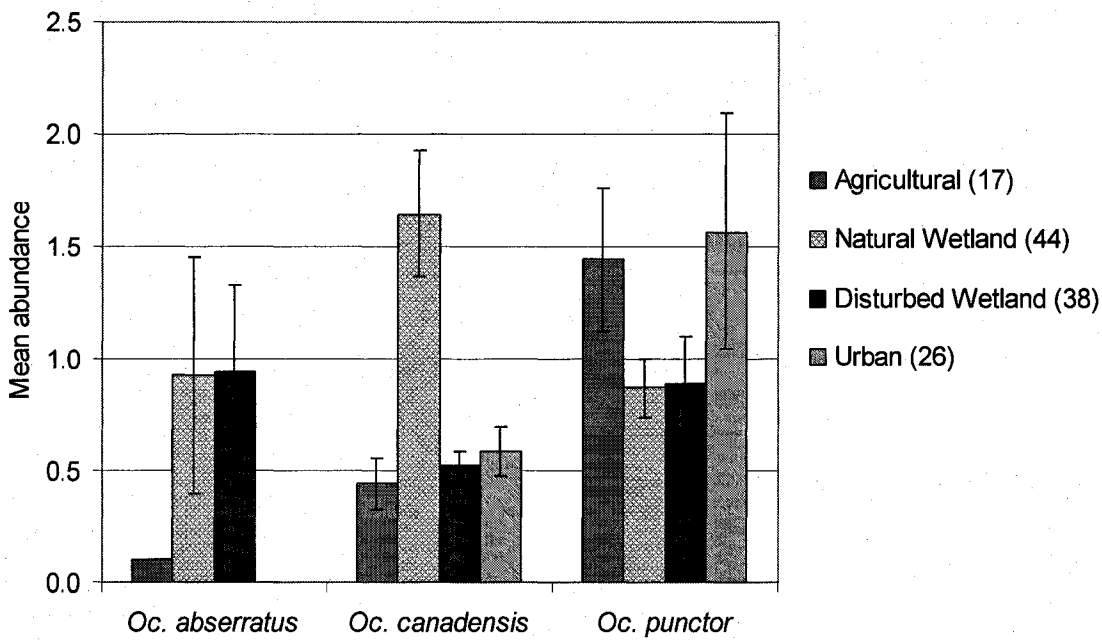


Figure 4.4 Mean abundances (measured as individuals per dip) of the three most abundant species of mosquito larvae identified from land-use categories (n) in St. John's, Eastern Newfoundland, 2004-2005.

4.3.3 Mosquito larvae of Western Newfoundland

Among 17 species of larvae identified in Western Newfoundland, *Cx. territans*, *Oc. canadensis*, *Oc. communis*, and *Oc. punctor* each accounted for over five percent of the total abundance of larvae collected. There was a significant interaction ($p= 0.0084$) in testing the frequency of occurrence of these four species together between habitat types and developed areas in this region. Figure 4.5 shows that overall, these larvae occurred most often in ditches in Cormack and Deer Lake and marsh/swamps in Corner Brook. Larvae collected from Cormack were found only in ditches and temporary pools. Table 4.5 summarizes the frequency of occurrence and abundances of larvae in the developed areas. A significant interaction was also found in the frequency of occurrence of the four most abundant species between towns ($p= 0.0025$). *Culex territans* and *Oc. canadensis*

were found most often in Deer Lake followed by Corner Brook however, *Oc. communis* and *Oc. punctor* were found most often in Deer Lake and Cormack (Table 4.4). When analyzed separately there were significant differences in the frequency of occurrence of *Cx. territans* ($p= 0.0068$) and *Oc. communis* ($p=0.0357$) in developed areas.

It can be seen from Table 4.5 that all species of larvae occurred most often in ditches and temporary pools; *Oc. canadensis*, *Oc. communis*, and *Oc. punctor* each showed the same trend. However, *Cx. territans* differed significantly ($p= <0.0001$) in its frequency of occurrence between habitats and occurred most often ditches and marsh/swamps. A significant interaction ($p=0.0010$) also resulted when these four species were compared between land-use categories. Figure 4.6 shows *Cx. territans* and *Oc. canadensis* were found the most often in urban sites compared to *Oc. communis* and *Oc. punctor* in agricultural sites. When analyzed separately, *Cx. territans* and *Oc. communis* were significantly different between land-use categories, $p= 0.0126$ and 0.0075 respectively, however there were no significant differences for *Oc. canadensis* and *Oc. punctor*.

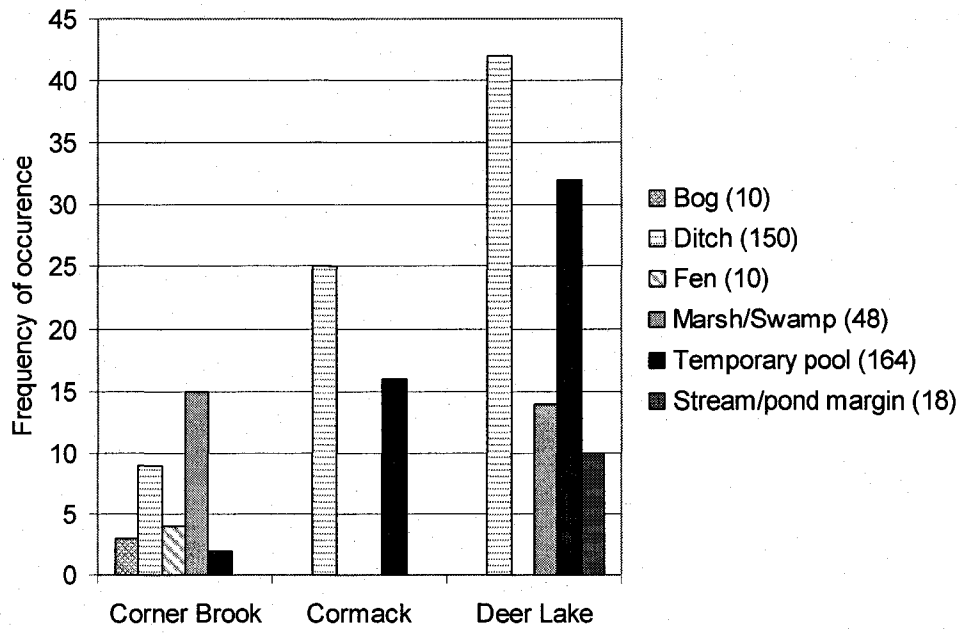


Figure 4.5 Cumulative frequency of occurrence of the four most abundant species of mosquito larvae in habitat types (n) identified from developed areas in Western Newfoundland, 2004-2005.

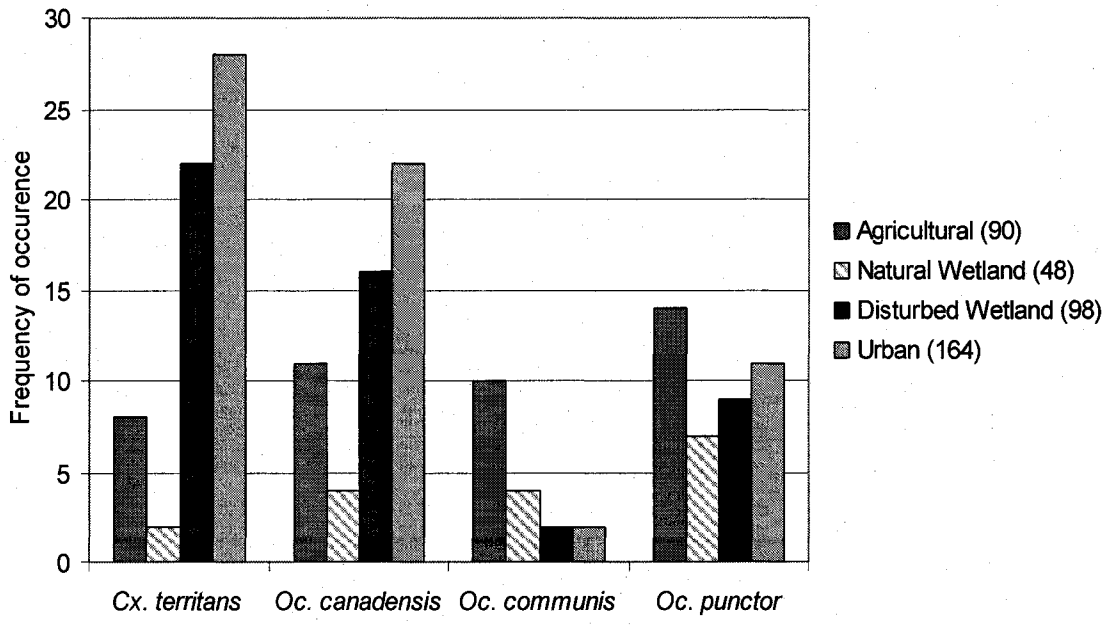


Figure 4.6 Cumulative frequency of occurrence of the four most abundant species of mosquito larvae identified from land-use categories (n) in Western Newfoundland, 2004-2005.

Table 4.4 Cumulative frequency of occurrence and mean abundance (measured as numbers per dip) of the four most abundant species of mosquito larvae and total larvae identified between developed areas in Western Newfoundland, 2004-2005 (SD= standard deviation).

	Cumulative frequency of occurrence				Mean abundance (SD)			
	Corner Brook	Cormack	Deer Lake		Corner Brook	Cormack	Deer Lake	
n	80	110	210	p-value	33	32	90	p-value
Cx. territans	13	6	41	0.0068	0.7 (0.7)	1.2 (0.7)	1.9 (1.4)	0.0022
Oc. canadensis	11	10	32	0.3091	1.2 (2.3)	3.9 (5.4)	0.8 (1.1)	0.0021
Oc. communis	2	10	6	0.0357	0.5 (0.1)	3.4 (6.6)	13.8 (22.7)	0.1580
Oc. punctor	7	15	19	0.3924	2.1 (2.8)	1.0 (0.7)	0.5 (0.4)	0.0012
Total species	33	32	90	0.4399	0.9 (1.6)	1.9 (3.7)	1.6 (5.2)	0.4470

Table 4.5 Cumulative frequency of occurrence and mean abundance (measured as numbers per dip) of the four most abundant species of mosquito larvae and total larvae identified between habitat types in developed areas of Western Newfoundland, 2004-2005 (SD= standard deviation).

	Cumulative frequency of occurrence						
	Bog	Ditch	Fen	Marsh/Swamp	Temporary pool	Stream/pond margin	
n	10	150	10	48	164	18	p-value
Cx. territans	1	29	1	18	7	4	<0.0001
Oc. canadensis	2	18	2	7	22	2	0.9517
Oc. communis	0	10	0	0	6	2	n/a
Oc. punctor	0	19	1	4	15	2	n/a
Total species	3	62	6	28	47	9	0.0009
	Mean abundance (SD)						
	Bog	Ditch	Fen	Marsh/Swamp	Temporary pool	Stream/pond margin	
n	3	62	6	28	47	9	p-value
Cx. territans	0.2 (0.0)	1.1 (0.8)	0.8 (0.0)	2.4 (1.7)	1.2 (0.7)	2.9 (1.1)	0.0002
Oc. canadensis	0.4 (0.3)	1.4 (2.2)	0.4 (0.3)	0.5 (0.2)	2.2 (3.9)	0.3 (0.1)	0.4676
Oc. communis	0.0	0.4 (0.3)	0.0	0.0	18.1 (21.1)	2.6 (2.8)	<0.0001
Oc. punctor	0.0	1.2 (1.8)	1.1 (0.0)	0.9 (0.7)	0.7 (0.5)	0.3 (0.1)	0.7033
Total species	0.3 (0.2)	1.1 (1.6)	0.5 (0.4)	1.4 (1.5)	2.5 (7.3)	1.0 (1.3)	0.1030

Note: n/a refers to analysis not being applicable due to complete absence of individuals in a habitat type.

There was a significant interaction (p= 0.0043) between the mean abundance of the four most abundant species together between land-use categories and towns in the west. Figure 4.7 shows overall highest mean abundances of these larvae were from natural wetlands in Cormack and Deer Lake and agricultural sites in Corner Brook. A significant interaction was also found for the mean abundance of these species between towns (p= <0.0001). The highest abundances of *Cx. territans* and *Oc. communis* were in Deer Lake, followed by Cormack, however, *Oc. canadensis* and *Oc. punctor* were most abundant in Cormack and Corner Brook respectively (Table 4.4).

It can be seen from Table 4.5 that the highest mean abundances of the four most abundant species varied by habitat type which explains the significant interaction (p= <0.0001) between these factors. However, when analyzed separately, only *Cx. territans* and *Oc. communis* differed significantly by habitat type with highest mean abundances recorded in stream/pond margins and temporary pools respectively. As indicated in Figure 4.8, the most abundant larval species also differ significantly by land-use categories (p= <0.0001). *Ochlerotatus communis* is not represented in this figure but is most likely responsible for this result as its highest mean abundance in a land-use category is four times that of any other species, with a natural wetland being preferred (p= <0.0001). Mean abundances of *Oc. canadensis* and *Oc. punctor* were also significantly different by land-use categories (p= <0.0001 and 0.0370 respectively) with highest abundances in agricultural sites, and *Cx. territans* (p= 0.0009) in disturbed wetlands.

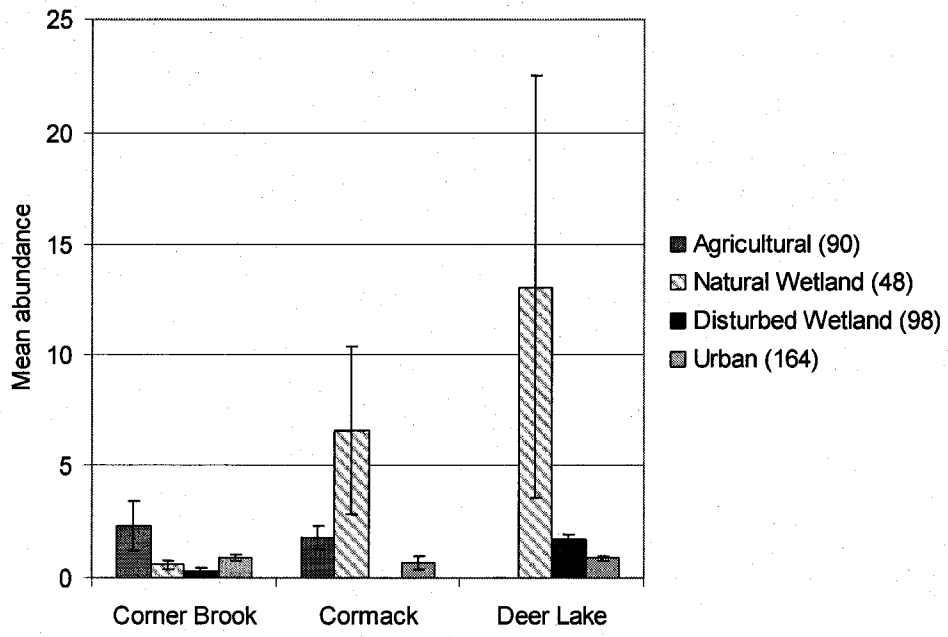


Figure 4.7 Mean abundances (measured as individuals per dip) of the four most abundant species of mosquito larvae combined in land-use categories (n) identified from developed areas in Western Newfoundland, 2004-2005.

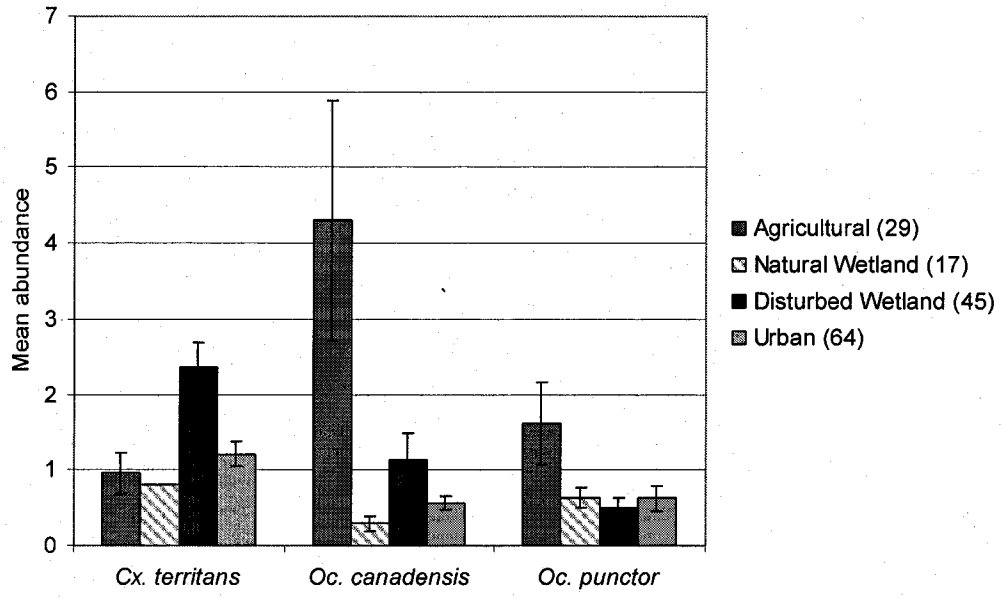


Figure 4.8 Mean abundances (measured as individuals per dip) of three most abundant species of mosquito larvae identified from land-use categories (n) in Western Newfoundland, 2004-2005.

4.3.4 Adult mosquito occurrence and abundance

4.3.4.1 Regional analysis of mosquito adult populations

Twenty-four species of adult mosquitoes were collected during the present study and their distributions are shown in Table 3.1 (Chapter 3). Among all adults identified, a significant difference was found in the frequency of occurrence ($p = <0.001$) and mean abundance ($p = 0.0015$) between eastern and western regions of the Island of Newfoundland (Table 4.6). More species occurred in the west, however mean abundance was higher in the east. Among those collected, nine species' total abundances each accounted for over five percent of the total mosquitoes collected, however, *Cx. pipiens* was collected only in the west, as shown in Table 4.6. Of those nine species, no significant differences in the occurrence of *Cs. minnesotae* and *Oc. canadensis* were found between regions. However, the mean abundance of *Cs. minnesotae* was significantly higher ($p = 0.0195$) in the west. Conversely, the mean abundance of *Oc. canadensis* was significantly higher ($p = 0.0004$) in the east.

Table 4.6 Cumulative frequency of occurrence and mean abundance (measured as number per positive trap) of the nine most abundant species of adult mosquitoes and total adults identified between Eastern and Western regions of the Island of Newfoundland, 2004-2005 (SD= standard deviation).

	Cumulative frequency of occurrence			Mean abundance (SD)		
	East	West		East	West	
n	248	160	p-value	186	131	p-value
Cq. perturbans	60	57	0.0131	9.5 (9.4)	8.7 (9.3)	0.6805
Cs. minnesotae	34	15	0.1912	2.0 (1.4)	3.8 (4.9)	0.0195
Cs. morsitans	77	27	0.0016	3.7 (3.5)	2.2 (2.8)	0.0120
Cx. pipiens	0	36	n/a	0.0 (0.0)	3.9 (4.2)	n/a
Oc. abserratus/punctor	92	39	0.0076	6.2 (7.4)	2.0 (1.7)	<0.0001
Oc. canadensis	113	77	0.6126	8.3 (8.2)	4.9 (5.7)	0.0004
Oc. communis	22	53	<0.0001	2.8 (3.4)	5.8 (6.5)	0.0059
Oc. diantaeus	7	31	<0.0001	1.1 (0.4)	5.7 (5.4)	0.0010
Oc. excrucians	44	49	0.0027	2.7 (3.0)	4.2 (3.6)	0.0183
Total species	186	131	<0.0001	0.7 (2.8)	0.6 (2.9)	0.0015

Note: n/a refers to analysis not being applicable due to absence of species in a region

Ochlerotatus abserratus/punctor and *Cs. morsitans* occurred significantly more frequently (p= 0.0076 and 0.0016 respectively) in the east compared to the west, whereas *Cq. perturbans* and *Oc. communis* were identified significantly more frequently (p= 0.0131 and <0.0001 respectively) in the west compared to the east. Significantly higher mean abundances were also found for *Oc. abserratus/punctor* (p= <0.0001) and *Cs. morsitans* (p= 0.0120) in the east compared to the west. In addition, there was a significantly higher mean abundance (p= 0.0059) for *Oc. communis* in the west compared to the east and there was no significant difference in the mean abundance of *Cq. perturbans* between regions.

4.3.4.2 Adult mosquito trapping transects analysis

Among 12 species of adults identified in St. John's, overall frequency of occurrence differed significantly (p= <0.001) between site groups (Table 4.7). Location of trapping sites from 1 to 10 begins on the outer fridges of the city progressing through the center of urban development and again through suburban surroundings (Figure 2.1; Chapter 2). No significant difference was found between overall mean abundance among site groups. Higher frequency of occurrences and significantly different mean abundances (p= 0.0003) of adult females did follow a trend and were located in site groups 1-3, and 8-10 (Table 4.7). Mean abundance of adult female mosquitoes across sites within the St. John's adult trapping transect is shown in Figure 4.9. Higher mean abundances in general were found at these peripheral locations, although the highest recorded mean abundance for an individual site was at site 4 in Lundrigan's Marsh, a *Cq. perturbans* breeding hot spot, and lowest at site 5, in the city core (Figure 4.9).

Table 4.7 Cumulative frequency of occurrence and mean abundance (measured as numbers per positive trap) of five most abundant species of adult mosquitoes and total adults identified among site groups in St. John's, 2004-2005 (SD= standard deviation).

	Cumulative frequency of occurrence				**Mean abundance (SD)**			
	Sites 1-3	Sites 4-7	Sites 8-10		Sites 1-3	Sites 4-7	Sites 8-10	
n	58	72	64	p-value	46	42	49	p-value
Cq. perturbans	10	23	5	0.0032	2.3 (5.6)	8.0 (11.1)	0.2 (0.7)	0.0056
Cs. morsitans	18	18	30	0.0251	1.5 (3.1)	1.7 (3.2)	2.2 (3.1)	0.9355
Oc. abserratus/punctor	25	11	24	0.0018	1.8 (4.2)	0.6 (1.3)	3.3 (6.6)	0.0120
Oc. canadensis	34	15	28	<0.0001	4.3 (5.7)	1.8 (3.6)	7.0 (9.9)	0.0023
Oc. excrucians	15	3	14	0.0062	1.1 (2.6)	0.3 (1.2)	0.9 (2.1)	0.7551
Total species	46	42	49	<0.0001	4.7 (5.5)	6.8 (8.3)	5.8 (7.6)	0.0713

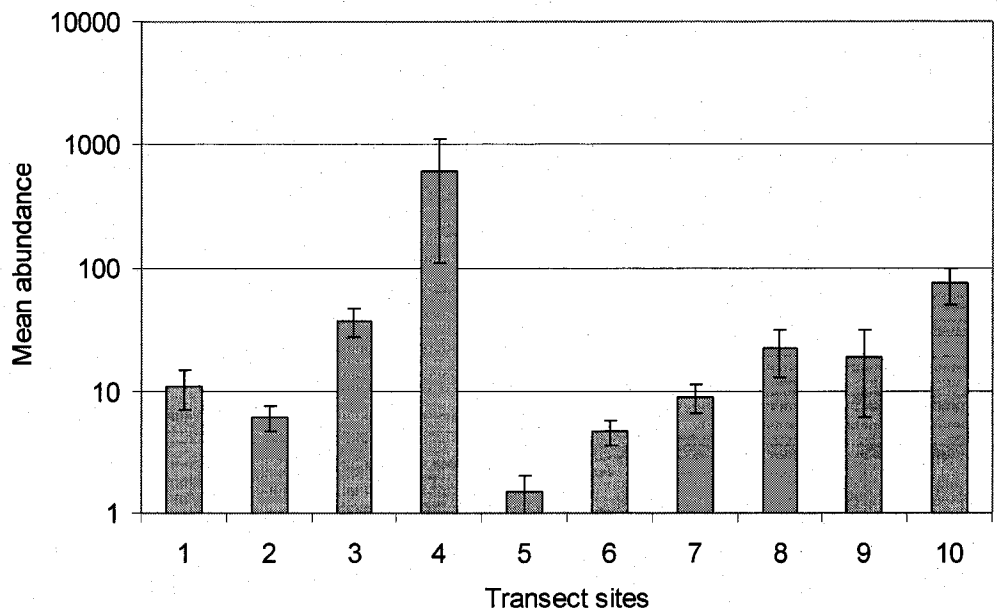


Figure 4.9 Distribution of the mean abundance (measured as number per positive trap) of adult female mosquitoes from transect sites in St. John's, 2004-2005.

Among those species collected in St. John's, total abundances of five species were each responsible for more than five percent of the total and all showed significant differences in their frequency of occurrence between site groups (Table 4.7). *Ochlerotatus abserratus/punctor*, *Oc. canadensis*, and *Oc. excrucians* occurred most often in sites 1-3, while *Cq. perturbans* occurred most often in sites 4-7, and *Cs. morsitans* in sites 8-10 (Table 4.7). There were no significant differences in the mean abundances of *Cs. morsitans* and *Oc. excrucians* between site groups. However, significant differences in the mean abundances of *Ochlerotatus abserratus/punctor* ($p= 0.0120$), *Oc. canadensis* ($p= 0.0023$), and *Cq. perturbans* ($p= 0.0056$) were found between site groups. *Cs. morsitans*, *Oc. abserratus/punctor*, and *Oc. canadensis* were

most abundant in sites 8-10, whereas *Cq. perturbans* was found to be most abundant in sites 4-7, and *Oc. excrucians* in sites 1-3 (Table 4.7).

Among 19 species of adult mosquitoes identified in Deer Lake, nine species had total abundances each making up over five percent of the total mosquitoes collected. These species included: *Cq. perturbans, Cx. pipiens*, *Oc. canadensis*, *Oc. communis*, *Oc. diantaeus*, *Oc. excrucians*, *Oc. fitchii*, and *Oc. pullatus*. A significant difference (p= 0.030) in the mean abundance of adult female mosquitoes among sites across Deer Lake was found. Sites 3, 7, and 8 were the most productive sites in Deer Lake and sites 4-6 yielded the lowest mean abundance of adult females (Figure 4.10). Overall frequency of occurrence of identified adults was not significantly different between sites. Also, when the frequency of occurrence and mean abundance of the nine most abundant species was compared between sites, there were no significant differences.

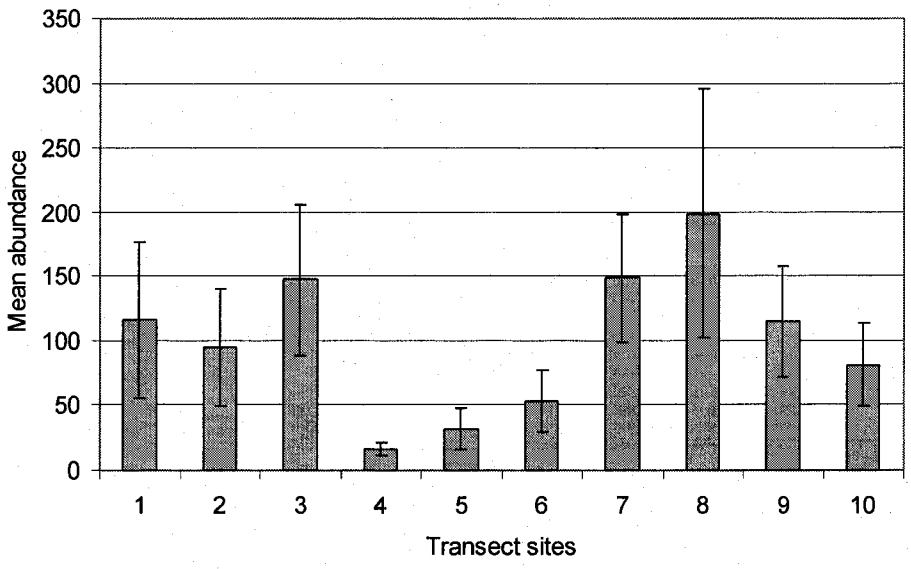
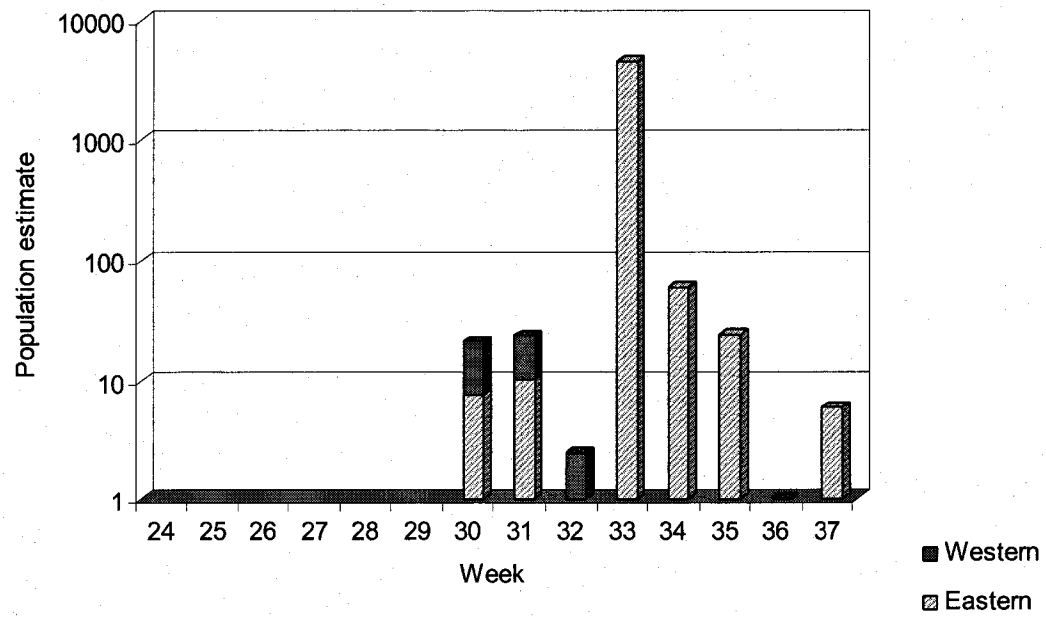


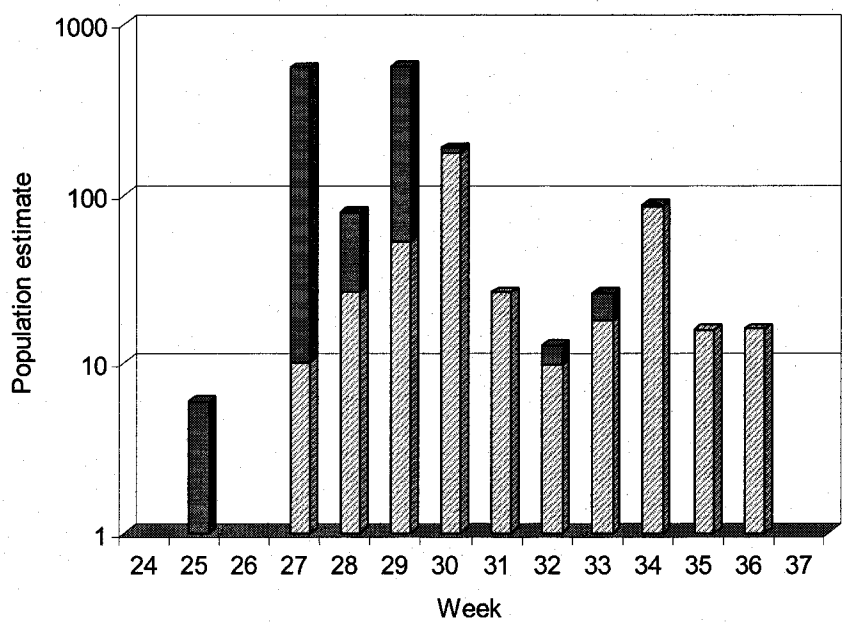
Figure 4.10 Distribution of the mean abundance (measured as number per positive trap) of adult female mosquitoes from transect sites in Deer Lake, 2004-2005.

4.3.4.3 Seasonal distribution of adult female Coquillettidia perturbans and Culex pipiens

Adult female *Cq. perturbans* were found island-wide in all trapping sites in Corner Brook, Cormack, and Deer Lake, within three parks each in the western and eastern regions, and in nine sites within St. John's. To evaluate the seasonal abundance of an island-wide species, the proportion of *Cq. perturbans* in an identified sample was multiplied by the total number of females trapped. When the seasonal abundance of *Cq. perturbans* between regions was analyzed, there was no significant interaction ($p = 0.9154$) between its population estimate and region in 2005. However, it was evident that the seasonal abundance of this species exhibits a different trend when regions are compared, as shown in Figure 4.11. *Coquillettidia perturbans* first appeared in week 30 in St. John's in 2004 and week 27 in 2005. Limited trapping weeks in the west in 2004 did not indicate onset of adult activity however, in 2005 this species appeared in week 25 in the west and was not trapped after week 34 (Figure 4.11).



a)



b)

Figure 4.11 Seasonal distribution of the estimated population abundance (measured as number per positive trap) of *Coquillettidia perturbans* in eastern and western Newfoundland in a) 2004 and b) 2005.

This seasonal difference was also evident in 2005 when only the urban areas of Deer Lake and St. John's were compared (Figure 4.12) although there was no significant difference (p= 0.0756) in the seasonal distribution of *Cq. perturbans* between these cities. Individuals were trapped in Deer Lake in week 25, three weeks prior to this species' appearance in St. John's in week 28. Furthermore, individuals were trapped in St. John's in week 36, two weeks after they were no longer collected in Deer Lake.

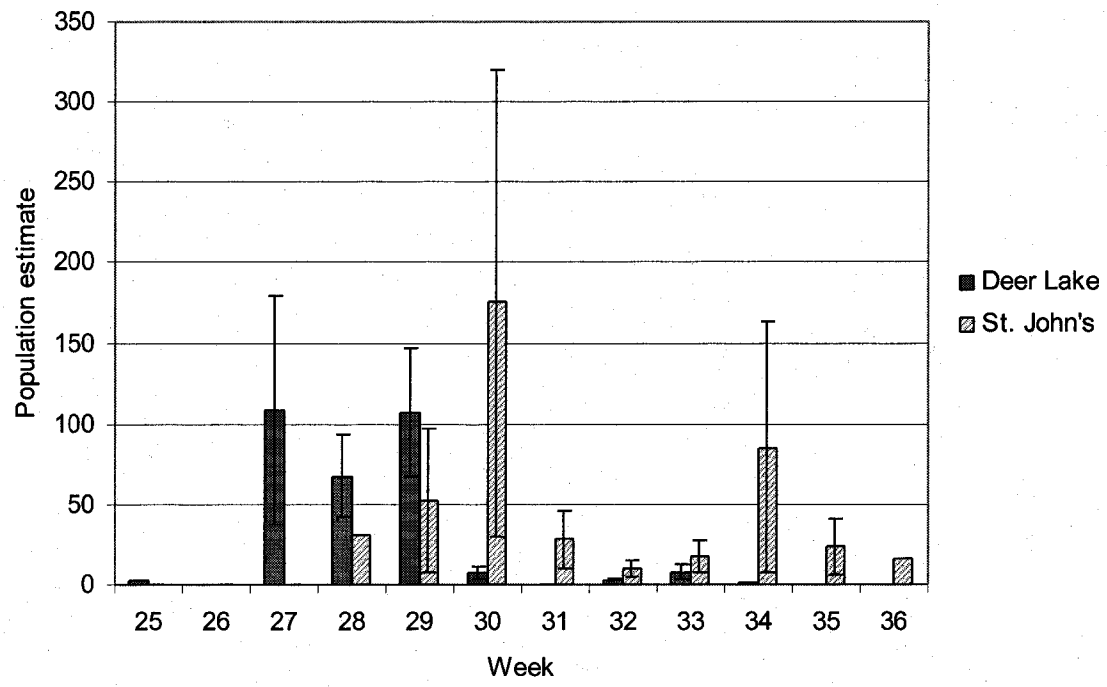


Figure 4.12 Seasonal distribution of the estimated population abundance (measured as number per positive trap) of *Coquillettidia perturbans* in Deer Lake and St. John's, 2005.

To test whether the seasonal abundance of *Cq. perturbans* was influenced by air temperature, an interaction between the mean abundance of this species in 2005 from Lundrigan's Marsh, a site within the city of St. John's where 70 percent of this species

was trapped, and temperature was analyzed. There was a significant difference between the trend of mean temperature and abundance of this species over the season (p=0.0315). However, Figure 4.13 indicates this species appeared only after significant increases in air temperature prior to trapping.

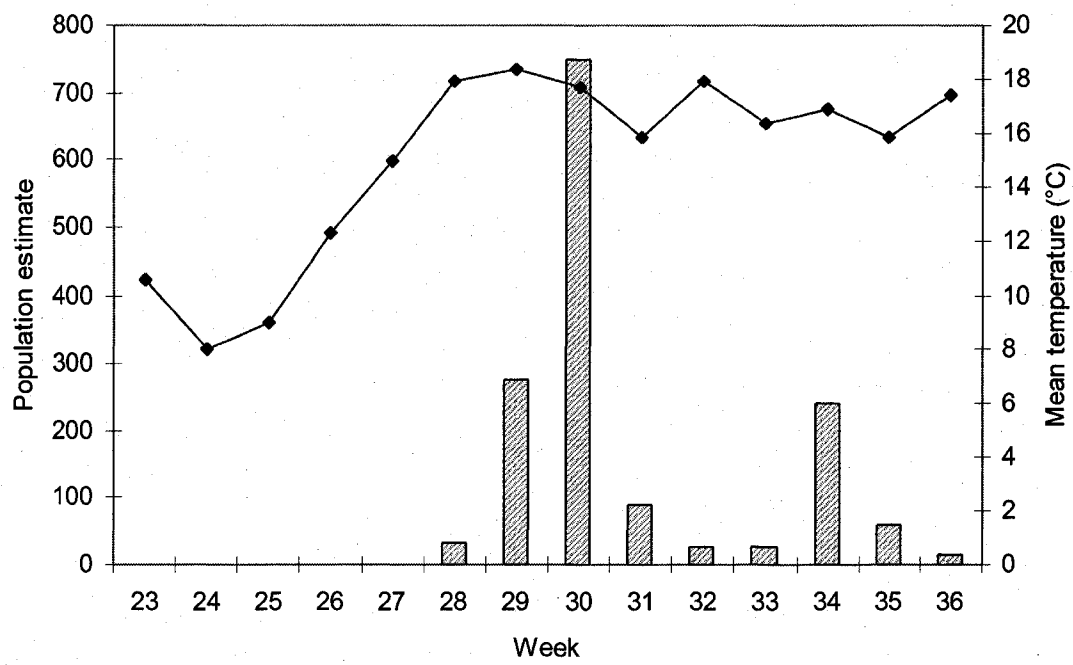


Figure 4.13 Seasonal distribution of the estimated population abundance (measured as number per positive trap) of *Coquillettidia perturbans* and mean air temperature one week prior to trapping in Lundrigan's Marsh, St. John's, 2005.

The seasonal distribution of *Cq. perturbans* in Deer Lake in 2005 is shown in Figure 4.14. An interaction between mean air temperature prior to trapping and estimated population abundance of this species in Deer Lake was insignificant (p= 0.4865), indicating these two factors appear to have the same trend over the season. This analysis was significant (p=0.0230) for *Cx. pipiens* in Deer Lake in 2005, indicating there

was some difference between the trend of these factors, although Figure 4.15 does show *Cx. pipiens* increasing in number after increases in air temperature prior to trapping.

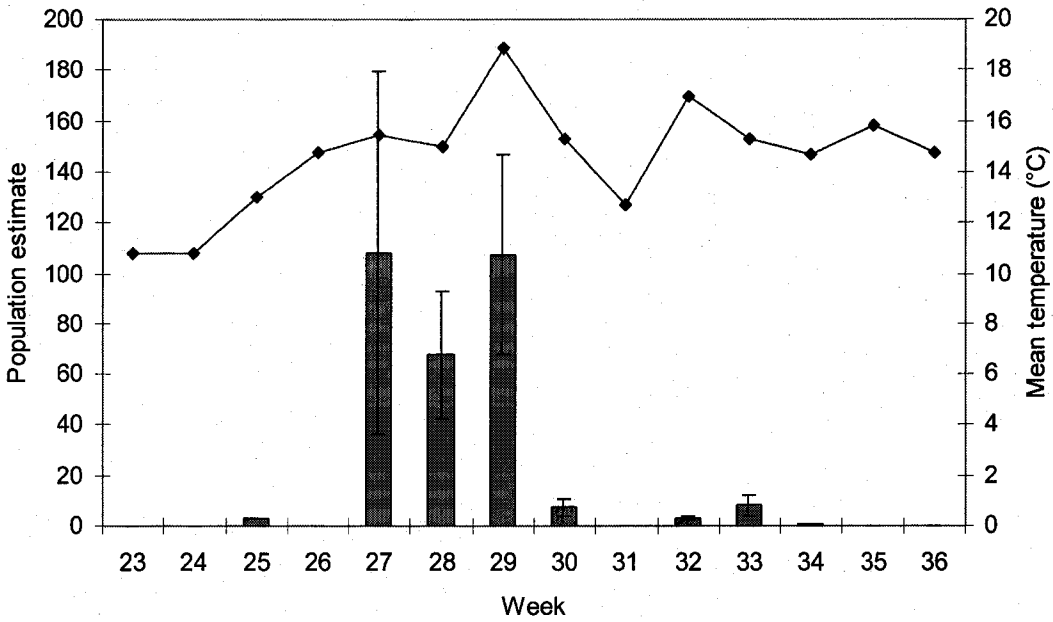


Figure 4.14 Seasonal distribution of the estimated population abundance (measured as number per positive trap) of *Coquillettidia perturbans* and mean air temperature one week prior to trapping in Deer Lake 2005.

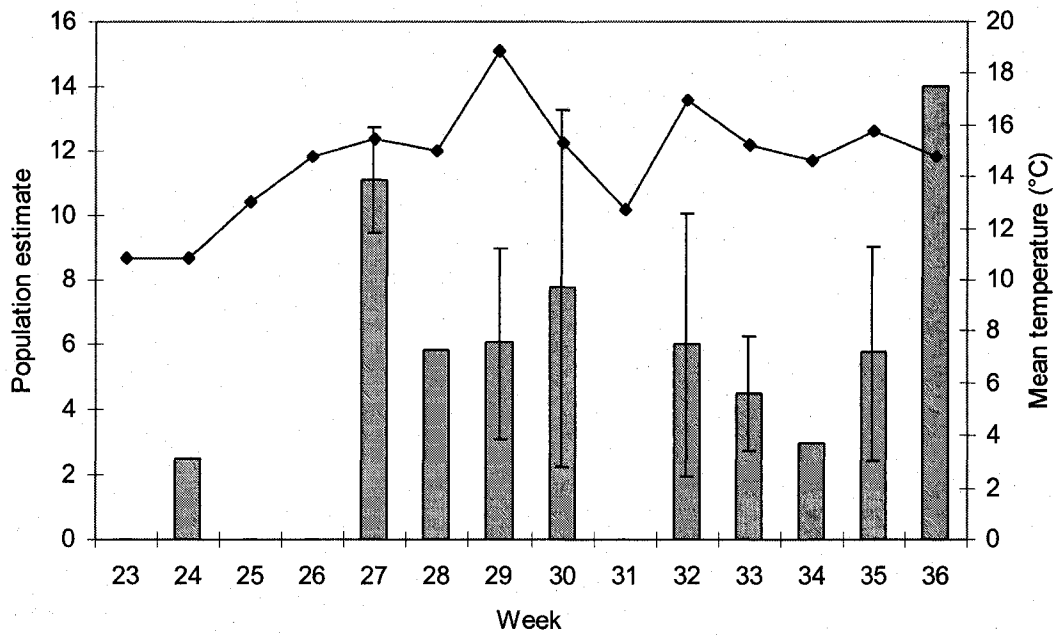


Figure 4.15 Seasonal distribution of the estimated population abundance (measured as number per positive trap) of *Culex pipiens* and mean air temperature one week prior to trapping in Deer Lake 2005.

4.4 Discussion

The current study showed the diversity of habitats used as breeding sites for mosquitoes in various land-use practices. These results support those of other studies which investigated breeding habitats and reveaed similar habitats are utilized by the mosquito fauna (Kronenwetter-Koepel et al. 2005, Joy and Clay 2002, Nielsen and Mokry 1982b, Lewis and Bennett 1979). No unusual habitats, i.e. tree holes, were found to be used on the Island of Newfoundland. It is possible that other habitats across the province are occupied by mosquitoes. This research did not exhaustively collect all aquatic habitats but rather concentrated on identified likely breeding locations in obvious standing water and monitored these over time. For example, the rocky splash pools along

the ocean and rivers were not sampled although Nielsen and Mokry (1982b) located mosquitoes in these habitats on the island. In addition, in order to evaluate the interaction of identified species and humans or domestic animals, the majority of the habitats sampled were located within or adjacent to populated centres and farms.

Many common species of mosquitoes occurred more frequently in the western region of the island compared to the east; a higher diversity of species was also seen in this region of the province. Environmental conditions influence populations in each region, and as such may be reflected in the diversity of the area. The western region of the island does have local climatic and habitat differences from the east, most notably its favourable growing conditions created by a relatively longer vegetative season and a generally warm summer (Damman 1983). Furthermore, the sampled areas in the west were characterized by a predominate forest of *Abies balsamea* and densely vegetated alluvial alder swamps whereas the barrens ecoregion, in which St. John's is situated, is dominated more by dwarf shrub heaths, bogs, and shallow fens (Damman 1983). This difference was clearly reflected in the greater occurrence of larvae in bog and fen habitats in the east. This study focused sampling efforts in the east in the city of St. John's, a much larger city than Deer Lake and surrounding areas, and as such, may explain differences between these regions. The western region will therefore have a higher proportion of undeveloped land compared to developed areas for urban or agricultural purposes.

The low human population on the Island of Newfoundland compared to other provinces allows for large expanses of natural forested or wetland habitat. It was in these

naturally occurring water basins in which the majority of mosquitoes breed. Temporary pools, classified here as ground pools or depressions which fill with water by snowmelt or precipitation events, most often represent these natural breeding sites with high abundances of mosquitoes. The use of these habitats for mosquito breeding was not surprising since the majority of the fauna was comprised of *Aedes/Ochlerotatus* species, thus hatching was closely related to snowmelt (Wood et al. 1979). These breeding habitats, in relation to cities such as St. John's and Corner Brook, were located mostly on the outer fringes of the cities; they may be modified to some extent but still hold many of their natural elements. In addition, ditches, whether created from agricultural or urban practices, were used frequently and harboured high abundances of larvae across the island. The creation of these habitats near populated areas increases the potential for mosquito contact with humans and domestic animals. This is of particular interest when it comes to vectors of disease, such as *Cx. pipiens,* an urban species that breeds in organically enriched habitats, and the transmission of WNV (Kronenwetter-Koepel et al. 2005).

It is expected that larvae of common species such as *Oc. canadensis*, *Oc. communis*, *Oc. punctor*, and *Oc. excrucians* will be found in diverse habitats (Wood et al. 1979), which are observed in Newfoundland since these species are distributed throughout the boreal forest and surrounding sphagnum bogs and grassy marshes. However, there are several instances in which habitats created in agricultural or urban influenced locations produced significantly higher abundances of these species. This was seen in populations of *Oc. punctor* in St. John's and *Cx. territans* and *Oc. communis* in the west. However,

the presence and abundance of *Cx. territans* were closely related to its amphibian hosts; it was in these habitats in which high abundances of frogs were observed. *Ochlerotatus communis* larvae aggregate in dense masses in response to certain combinations of light and temperature conditions (Wood et al. 1979) which could skew results if this behaviour is found in a sampling location.

Several habitats created by urbanization and agricultural practices which have had an increase in nutrient load from these practices were not breeding a diversity of mosquitoes. This was seen in Lundrigan's Marsh in St. John's in which a high abundance of *Cq. perturbans* was collected as adults, but other larval species were not found. Lundrigan's Marsh is a fen which was converted to a cattail marsh, probably as a result of nutrient load from surrounding businesses as well as runoff from a nearby dumpsite. Interestingly, a somewhat natural fen a few hundred meters from this marsh was inhabited by several species of mosquito larvae. This was also observed in Cormack where few or no larvae were collected from heavily polluted water bodies, however nearby habitats with little disturbance were productive.

It was clear from the adult trapping transects in St. John's that mosquitoes are not moving into the city but are in higher abundance on the fringes. This may be due to the higher proportion of natural breeding habitat surrounding the city and lack of breeding habitats in the urban areas, in part due to the soil being dominated by coarse glacial till that does not readily hold water. Furthermore, a common occurrence in urban centres elsewhere is high abundances of container breeding mosquito species such as *Cx. pipiens*. Habitats such as catch basin drainage systems and water holding containers within

residential yards are potential breeding sites for such species and can allow them to evade natural predators (Washburn 1995). However, sustained high temperatures elsewhere allow mosquitoes to develop at a faster rate and survive periodic higher volumes of water flow. These temperatures are not seen in Newfoundland and catchment breeding mosquito species are not found in St. John's.

Although not significant, this same trend was seen in the smaller town of Deer Lake. However, it is important to remember that no two traps in Deer Lake were more than two and a half kilometres apart. Although the three traps located in the more centralized part of town had the lowest catches, these results may be artificial due to the influence of external light sources, as this is known to skew the results of light trap data as lights may diminish the attractiveness of the trap (Service 1993a). Other sites in Deer Lake with the highest catches may have been a reflection of more forested habitat around them that reduced the impact of additional light. In reality, mosquito populations probably do not differ all that much between sites in this town.

Few breeding sites in high intensity urban centres, where the majority of natural land cover has been disturbed, is due to human-made materials and coarse textured soils that are impermeable or retain little standing water. There are catch basins and containers, and although catch basins were not sampled, the evidence from adult samples suggests these habitats are not used extensively as container breeding mosquitoes were not in abundance. A low abundance of adult mosquitoes in the city of St. John's may be a reflection of this lack of standing water in built up areas, and it was only in low intensity urban sites adjacent to forests and wetlands that large numbers of adults and

larvae were found. Flood control drainage ditches and wetlands were the most productive breeding habitats in these less populated locations.

Over the past three decades, development in St. John's and other urban centres has seen a marked increase which has changed the surrounding landscape. Although the current study showed urban centres have fewer mosquitoes, many people in these centres live in less populated developments surrounding these centres, thus exposed to a greater diversity and abundance of mosquitoes than in the true urban core. Furthermore, Statistics Canada, Census of Agriculture, report changes increases in crop-based agricultural land-use. Increased fertilizer and pesticide use is often associated with such practice, and these expanses of land commonly provide drainage ditches and temporal wetlands which are being used extensively by the mosquito fauna. This increases the availability of nutrient-rich habitat and potentially exposes mosquitoes to pesticide contamination. These developments have the potential to change mosquito populations. It is interesting that this study found *Cx. pipiens* here for the first time and although it may have been here previously, changes in land use may have increased its populations as this species prefers enriched habitats (Wood et al. 1979).

It is important to note that although human modified habitat does not always initially relate specifically to a vector, it could influence or enhance animal populations that have the potential to be hosts of a pathogen. Although not a virally significant species, an example of this is *Cx. territans* and the movement of its amphibian hosts. The creation of appropriate habitat for introduced frogs may have allowed frog populations to reach a level able to sustain a population of *Cx. territans*, as this species was previously

known from Labrador where frogs are native. This principle can also be applied to bird populations such as crows and their increasing populations in suburban and urban areas (Yaremych et al. 2004). Abundant and easily located anthropogenic food resources in urban and agricultural habitats are being exploited by these bird populations resulting in a diminishing need for forest food resources. The preference for crows and other avian species for urban habitat may expose these birds to the urban-dwelling *Cx. pipiens*, the primary vector of WNV in eastern Canada (Drebot et al. 2003).

Although corvids are highly susceptible to WNV, other avian species occurring in these habitats are also of concern since they have the potential to serve as reservoir hosts, such as the House Finch (*Carpodacus mexicanus* (Muller)), House Sparrow (*Passer domesticus* Linnaeus), and American Robin (*Turdus migratorius* Linnaeus) (Kilpatrick et al. 2006, Komar et al. 2003). In addition, mosquito species within these areas also have cattle, horses, and other domestic animals as potential hosts. The availability of breeding habitat and hosts in these areas increases the potential for mosquito-human contact and pathogen transmission.

In summary, the data presented clearly showed a seasonal difference in the emergence and adult activity between the western and eastern regions of the island. Estimated mosquito populations in the west appear to be active as much as three weeks prior to the east and end approximately two weeks earlier. Although a warm summer is characteristic of the western region, lower mean temperatures over the season compared to St. John's are prevalent. This difference can be explained by the fact that Deer Lake is an interior location, distant from the pronounced modifying influence of the North

Atlantic seen in St. John's. Although the west has lower monthly mean minimum temperatures, it has higher monthly mean maximum temperatures compared to St. John's. With respect to larval development and adult mosquito activity, sunshine is critical, and maximum daily temperatures with more sunshine and less oceanic fog are more important to warm pools in larval habitats.

Culex pipiens activity in Deer Lake was first noted in the middle of June, week 24; these individuals were probably overwintering females and an increase in adult activity occurred after the oviposition of these individuals. This species can have several generations over the season and if adult trapping had continued beyond the middle of September, week 37, it is expected that more individuals would have been captured since breeding continues until it is diminished by cold weather in the fall (Spielman 2001, Wood et al. 1979). Now that this species is known to occur here and a few larvae were found, interestingly not in overly enriched breeding sources, a more focused study is needed. Intensive larval sampling in the western region to locate the dominant breeding habitat of this species and evaluate the contribution of catchment basins in their development, as well as adult sampling using gravid traps, is a logical next step to clearly define the distribution and population levels of this important vector.

5 WEST NILE VIRUS SURVEILLANCE AND POTENTIAL TRANSMISSION IN NEWFOUNDLAND

5.1 Introduction

Arbovirus studies involving mosquitoes in Newfoundland have been limited. However, recognition of potential health risks associated with biting flies and clinical cases of California (CAL) serogroup viruses as far east as Nova Scotia, and antibody detection in several animal groups around the Maritimes, prompted research to determine the eastern limit of abrovirus distribution in North America. Studies performed in Newfoundland from 1980 to 1983 resulted in the isolation of three CAL viruses including an isolate of Jamestown Canyon (JC) virus from a mixed pool of *Oc. abserratus* and *Oc. punctor* and two isolates of snowshoe hare (SSH) virus from pools of *Oc. canadensis* (Mokry et al. 1984). In addition, serological studies on human, horse, and snowshoe hare (*Lepus americanus*) sera confirmed the circulation of JC and SSH viruses in Newfoundland (Mokry et al. 1984).

Furthermore, a study to examine the reproductive and vector potential of four major Newfoundland *Aedes/Ochlerotatus* species was also going on at this time. Season-long sampling of the adult population, determining reproductive capacity, and physiological age-grading suggested that no more than about 25 percent of adults return for a second blood-meal and less than five percent return for a third (Mokry 1984). The epidemiological significance of this to the transmission of CAL viruses is that potentially infected females would not live long enough to refeed and infect further hosts.

Laboratory confirmed cases of WNV disease observed during an outbreak in the summer of 1999 in New York City were the first in the Western hemisphere (White et al. 2001, Nash et al. 2001). The incursion of WNV into Canada was documented for the first time in 2001 in Ontario. By the end of 2005, this virus had expanded its geographical range in North America with documented activity in 48 American states, and seven Canadian provinces (excluding British Columbia, Prince Edward Island, and Newfoundland and Labrador) (Artsob et al. 2006).

After the detection of WNV, the goal of mosquito surveillance in Canada was to gain a better understanding of the specific role that different species play in the enzootic and epidemic transmission of this disease and to use this information to guide decision making concerning the need to implement mosquito control strategies. Species composition, relative abundance, and seasonal patterns of activity of adult mosquitoes in different at-risk localities were to be determined, and collected mosquitoes were tested for evidence of WNV infection. To date, WNV has been detected in 14 different mosquito species (Artsob et al. 2006, Drebot et al. 2003) including *Cx. pipiens*, the primary enzootic vector in North America, which has been shown to maintain the virus through the winter (Artsob et al. 2003).

The incursion of WNV into Canada has recently prompted mosquito surveillance in Newfoundland. As a result of this study, three additional species were found including, *Cq. perturbans*, *Oc. provocans*, and *Cx. pipiens*. These results are summarized in Chapter 3. No reported vectors of WNV were known to occur in Newfoundland until this. The focus of surveillance in Canada has also been on birds, horses (or other

domestic animals) and humans. Corvidae (e.g. crows, blue jays, ravens) appear to be particularly susceptible to mortality following exposure to WNV (Komar et al. 2003, Eidson et al. 2001). As such, corvids, crows in particular, have been the primary target species for dead bird surveillance programs in North America as an early indicator of virus activity in a local ecosystem. In addition, although other bird species are likely to be affected by WNV, crows are relatively large-bodied, tend to be abundant in suburban and urban areas, and are likely to be seen by the general public. The Canadian Cooperative Wildlife Health Centre (CCWHC) organized a system for recording dead bird sightings and maintaining a database for tracking bird submissions and reporting results of diagnostic testing to provincial public health authorities. Surveillance for WNV in horses is often conducted to estimate the impact of infection on these populations and to serve as an indicator of virus activity in rural communities, where it is anticipated that, because of low human population density, dead bird surveillance might not be as effective compared to urban centres.

The objective of this investigation was to estimate the level of risk for human and animal exposure to potential WNV transmission in Newfoundland. In doing so, possible pathways of entry and potential sites of transmission are discussed.

5.2 Materials and Methods

Between May and October in 2004 and 2005, oropharyngeal swabs from field-collected dead corvids, submitted by local or provincial authorities, were tested using the VecTest antigen-capture assay. This assay was provided by the CCWHC and has been proven to be a reliable and rapid diagnostic test (Stone et al. 2005, Lindsay et al. 2003,

Yaremych et al. 2003). Results of these tests were then entered into the CCWHC national surveillance database.

An equine sera survey was performed to detect the presence of the virus through ELISA antibody screening. Upon consent from horse owners, provincial veterinarians obtained blood samples from horses in their respective health districts during the summer of 2004. In 2005, equine sampling efforts were focused in the Western health district following the discovery of *Cx. pipiens* in this region. Information about individual horses was obtained, including age and vaccination and travel history. Serum samples were sent to and tested by the Animal Health Laboratory, University of Guelph. When WNV IgG antibody capture by ELISA was positive, or horses were known to have histories of previous vaccination to WNV, these samples were then sent to either the Prairie Diagnostic Services, Western College of Veterinary Medicine, University of Saskatchewan; Animal Health Diagnostic Center, College of Veterinary Medicine, Cornell University; or the National Microbiology Laboratory, Winnipeg, for IgM antibody capture by ELISA and/or serum neutralization (SN), which would clarify if these individuals had recent exposure to the virus.

5.3 Results

Ninety-one corvids tested negative for WNV infection in 2004 and sixty-three in 2005. Of these 154 corvids, 145 were American Crows (*Corvus brachyrhynchos* Brehm), two Blue Jays (*Cyanocitta cristata* Linnaeus), and seven Common Ravens (*Corvus corax* Linnaeus). The majority of corvid deaths were attributed to electrocution.

The distribution of tested corvids is shown in Table 5.1. See Appendix 2 for a map of health and community services boards.

Table 5.1 Distribution of corvids tested for West Nile virus infection in Newfoundland and Labrador health and community services boards, 2004-2005.

	2004	2005
St. John's	52	24
Eastern	7	7
Central	11	11
Western	15	17
Grenfell	0	1
Labrador	6	3

A total of 52 horses were sampled in 2004 and all sera were negative for WNV. In 2005, a total of 50 horses were sampled. All but one tested negative to WNV. This serum was from a horse in the Western region of the province and was thought to have no vaccination history to WNV. The serum gave a negative IgM ELISA result and a positive SN result, as well as a positive hemagglutination inhibition (HI) assay to WN from a second sample. Such an outcome would suggest this horse had prior exposure to the virus due to vaccination or infection, causing concern since this area of the province was known to have an established vector population. Further probing into the horse's history of vaccinations revealed it had previously been vaccinated for WN which could explain the results obtained from the serum.

5.4 Discussion

For an arbovirus to be amplified successfully in nature and for an epizootic outbreak to occur in a region, several conditions must exist. The virus in question must be capable of replicating in a blood-feeding arthropod species, this species must then be

capable of transmitting the virus when taking a second blood meal, and its population density must be of appropriate size to maintain the virus. In addition, a susceptible vertebrate host(s) must be present, and during infection of a vertebrate host, the virus must be at a sufficient titre for an acceptable duration to infect a subsequent feeding arthropod (Artsob et al. 2006, Turell et al. 2002). Furthermore, proper temperature conditions must be met not only for the arthropod population's development but to aid in viral amplification within the arthropod and vertebrate hosts.

To date, no WNV activity has been documented in Newfoundland. However, a known competent vector of the disease, *Cx. pipiens*, is now known to occur in the western region of the island. Compared to other species in this region, the population of *Cx. pipiens* was low and this may limit its potential to become infected. Environmental factors, such as temperature and rainfall, have long been known to influence the transmission cycles of arboviruses. It has been shown that *Cx. pipiens* is a significantly more efficient vector of WNV when the mosquitoes are held at 30°C, than when they are held at or below 26°C (Dohm et al. 2002a). The ability of WN and other mosquito-borne pathogens to extend their geographical range and enhance their transmission rates is directly influenced by changes in climatic conditions (Reiter 2001, Gratz 1999, Reeves et al.1994). A daily mean temperature increase and changes in patterns of precipitation caused by global warming have the potential to influence the range of vectors and incidence and distribution of these diseases.

The specific roles of most of the known susceptible mosquito species in the WNV transmission cycle in North America are unknown, but several of these species appear to

be competent (Goddard et al. 2002, Turell et al. 2001, Sardelis et al. 2001). Although species in the genus *Culex* appear to be the main vectors implicated in the avian amplification cycle of WNV, with *Cx. pipiens* being important in eastern North America, a number of susceptible mosquitoes are opportunistic feeders. Several of these opportunistic species may be important bridge vectors that move the virus from the primary enzootic cycle between *Culex* and avian hosts to mammals, posing a much greater threat to humans and equines (Turell et al. 2002). Opportunistic species can include *Aedes*, such as *Ae. vexans*, which was collected only once in the province, *Ochlerotatus*, such as *Oc. canadensis*, an abundant species, other *Culex* species, and the widely distributed *Cq. perturbans*. (Kilpatrick et al. 2005, Turell et al. 2002, Sardelis et al. 2001). However, recent studies also show that *Cx. pipiens*, although not previously considered important in transmitting WNV to humans, is responsible for human infections in some regions due to shifts in their feeding behaviour from primarily birds to humans (Kilpatrick et al. 2006, Kilpatrick et al. 2005, Spielman 2001). Thus, species implicated elsewhere to be involved in the transmission cycle of WNV do occur on the Island of Newfoundland.

Birds play a central role in amplification cycles of WNV (Marra et al. 2004, Hubálek and Halouzka 1999, Komar 1999). As previously mentioned, high rates of mortality have been observed in some bird species (i.e. Corvidae) while other species may show little to no effects from exposure to WNV. In the laboratory, passerines such as Blue Jays (*Cyanocitta cristata*), Common Grackles (*Quiscalus quiscula* Linnaeus), House Finches (*Carpodacus mexicanus*), Americans Crows (*Corvus brachyrhynchos*),

and House Sparrows (*Passer domesticus*) appear to be highly competent reservoirs of 25 tested species, while other species are incompetent reservoirs (e.g. Rock Pigeons (*Columba livia* Gmelin), Ring-necked Pheasants (*Phasianus colchicus* Linnaeus), and Japanese Quail (*Coturnix japonicusa* Temminck and Schlegel) (Reisen et al. 2005, Komar et al. 2003). Artsob et al. (2006) stated that the importance of a given bird species to act as a reservoir of WNV depends in part on its susceptibility to infection, the proportion of vectors that become infected when feeding upon infected hosts, and the duration of infection (i.e. the number of days that an animal maintains an infectious viremia).

It has been shown that the introduction and observed patterns of WNV spread in the Western Hemisphere are best explained by migratory birds as critical long-distance transport agents (Peterson et al. 2003, Rappole et al. 2000). There is difficulty in determining the intensity and duration of viremia in naturally infected wild birds and the timing of migration and presence of a capable vector population. Nonetheless, migratory birds have long been suspected as critical agents in outbreaks of this disease and other arboviruses; under this view mosquitoes would then be responsible for enzootic transmission of the virus among hosts, as well as for spread on local scales. It should also be noted that although an individual migrant bird species may bring infection into a region, the pathogen may not become established.

Given the number of additional mosquito species found in Newfoundland for the first time in this study, there is a potential for additional species to colonize the province. In addition to potential colonization of infected vector species, there is evidence for

WNV to reach the island through the movement of avian populations along southeastern and circum-gulf migration patterns (Peterson et al. 2003). In conclusion, although no evidence of transmission of WNV was found, several additional mosquito species including an efficient vector are established here. Changes in land-use influencing certain types of larval habitats, increasing numbers of vertebrate hosts, such as domestic animals and those introduced by human activity, and climate change could play determining roles in the occurrence and/or establishment of WNV in Newfoundland and may increase the future risk of transmission. Therefore, an ongoing assessment of the potential risk of WNV and other arboviruses is prudent.

6 CONCLUSION

The present study found 22 previously collected mosquito species and three additional species: *Cq. perturbans*, *Cx. pipiens*, and *Oc. provocans*, representing seven genera. In addition, *An. earlei* and *Cx. territans* were previously collected only in Labrador but are now recorded on the Island of Newfoundland. Detection of these changes highlights the value of monitoring mosquito fauna over time, particularly when there is potential threat from a mosquito vectored arbovirus or other pathogen. The discovery of an established population of *Cx. pipiens*, although in low abundance, shows how risk assessment of a disease requires this increased knowledge.

It is clear that land-use changes due to anthropogenic practises can influence mosquito populations. Newfoundland's mosquito fauna used suitable habitat, such as ditches and temporary pools, in rural and agricultural areas where the intensity of these changes is greatest, particularly with regard to increased nutrient levels, although they maintained many characteristics of natural habitats. In addition, it is these impacted sites that crows and other potential hosts select for habitat use. Although container breeding in urban centres was not observed, habitats such as converted cattail marshes proved to be a significant source of the pest species, *Cq. perturbans*. Futhermore, the prime larval habitat of *Cx. pipens* and whether it was enhanced by human land-use practices was not elucidated here.

The change in land-use in the province with increased agriculture, urbanization, deforestation, and other development projects have the potential to result in changes in mosquito population densities and occurrences. These changes, in addition to altering

larval habitat, also change potential host populations that can enable the emergence of diseases such as WNV. Furthermore, predicted changes in climate towards intense periodic precipitation events and warmer weather will enhance evaporative enrichment of these habitats and influence the establishment of such pathogens. Although there has been a focus on the potential impact of climate change on human health with respect to mosquito-borne pathogens, the literature clearly shows that human activities and their impact on the local ecology have generally been much more significant in the prevalence and range of these diseases (Reiter 2001, Gratz 1999).

WNV has not been detected in Newfoundland and at this time, the level of risk to human and animal populations is low. However, as mentioned in Chapter 5, continued long-term monitoring of mosquito populations given changes in land-use and local climate is needed to maintain a confident predicted level of risk. These baseline data will enable us to better understand the ecology of potential diseases in a new geographical range, as was the case when WNV emerged in North America, from infected migratory birds entering Newfoundland. In addition, knowledge of mosquito species composition, relative abundance, breeding habitat, and seasonal trends can be used to assess the risk of other diseases as well as guide local response concerning the implementation of control activities if needed in the future.

Furthermore, the role of specific bird species to act as reservoir hosts varies across the range of WNV, partly because of the diversity of available avian species. Artsob et al. (2006) reported a recent study designed to determine if the incidence of WNV is more closely related to the abundance of vector mosquitoes or the diversity of bird

communities along urban-to-rural gradients. Only low bird diversity was an important predictor of WNV incidence in vector mosquitoes; based on a theory that a potential dilution effect may exist whereby a greater diversity of species includes more species that are poor amplifying hosts. These results suggest that efforts to conserve bird diversity by habitat conservation in urban and agricultural landscapes may actually contribute to WNV control efforts. These findings are of particular interest to Newfoundland where land-use changes are occurring, although not to the degree of other urban and agriculture locations across the country. Therefore, small changes in these human activities to limit the number of potential mosquito breeding habitats, while conserving avian species habitat, will significantly reduce the likelihood of a WNV outbreak should this disease be introduced.

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8 APPENDICES

8.1 Appendix 1: Frequency of occurrence and abundance of mosquito species collected in Newfoundland and Labrador 2004-2005.

Table 8.1 Frequency of occurrence of species of larval and adult mosquitoes collected in Newfoundland and Labrador, 2004-2005.

	Labrador	Western Parks	Corner Brook	Deer Lake	Cormack	Central Parks	Eastern Parks	St. John's
				LARVAE				
n	0	4	80	210	110	4	8	262
Ae. cinereus	0	2	2	11	5	2	3	9
An. earlei	0	0	1	0	0	0	0	0
Cs. impatiens	0	0	0	1	0	0	0	0
Cs. minnisotae	0	0	1	0	0	0	0	5
Cs. morsitans	0	1	6	9	1	0	2	18
Cx. pipiens	0	0	0	3	0	0	0	0
Cx. territans	0	0	13	41	6	0	0	0
Oc. abserratus	0	0	0	0	0	0	1	13
Oc. canadensis	0	4	11	32	10	2	5	65
Oc. cantator	0	0	0	0	0	0	0	2
Oc. communis	0	0	2	6	10	1	1	0
Oc. decticus	0	0	0	0	0	0	1	2
Oc. diantaeus	0	0	0	3	0	0	0	0
Oc. excrucians	0	0	1	1	2	0	0	7
Oc. fitchii	0	0	2	5	2	0	0	0
Oc. implicatus	0	0	0	3	4	0	0	0
Oc. intrudens	0	0	0	5	0	0	0	0
Oc. pionips	0	0	1	2	3	0	0	0
Oc. provocans	0	0	1	1	4	0	0	0
Oc. punctor	0	2	7	19	15	2	4	51
				ADULTS				
n	6	43	15	84	18	8	54	194
Ae. cinereus	0	1	0	22	5	4	6	7
An. earlei	0	0	0	4	0	1	0	0
Cq. perturbans	0	5	7	37	8	6	22	38
Cs. impatiens	1	2	1	1	0	0	1	0
Cs. melanura	0	0	0	0	0	0	3	5
Cs. minnesotae	0	6	2	7	0	0	13	21
Cs. morsitans	2	7	0	18	2	5	11	66
Cx. pipiens	0	0	2	32	2	0	0	0
Cx. territans	0	0	0	0	0	0	1	0
Oc. abserratus/punctor	1	14	3	16	6	7	32	60
Oc. canadensis	0	18	6	45	8	6	36	77
Oc. cantator	0	1	0	0	0	1	10	1
Oc. communis	0	4	3	32	14	2	15	7
Oc. decticus	0	0	0	1	0	0	2	0

Table 8.1 continued

	Labrador	Western Parks	Corner Brook	Deer Lake	Cormack	Central Parks	Eastern Parks	St. John's
				ADULTS				
n	6	43	15	84	18	8	54	194
Oc. diantaeus	0	1	0	30	0	0	7	0
Oc. excrucians	2	2	1	41	5	2	12	32
Oc. fitchii	0	1	0	40	3	0	0	0
Oc. implicatus	0	0	0	1	0	1	0	0
Oc. intrudens	0	0	0	14	0	0	6	0
Oc. nigripes	1	0	0	0	0	0	0	0
Oc. pionips	1	0	0	0	0	0	0	0
Oc. provocans	0	0	0	5	2	0	0	0
Oc. pullatus	0	0	0	9	1	0	0	0

Table 8.2 Cumulative abundance of species of larval (measured as number per dip) and adult mosquitoes collected in Newfoundland and Labrador, 2004-2005.

	Labrador	Western Parks	Corner Brook	Deer Lake	Cormack	Central Parks	Eastern Parks	St. John's
				LARVAE				
n	0	4	80	210	110	4	8	262
Ae. cinereus	0	0.6	1.0	4.2	9.9	0.8	1.6	3.9
An. earlei	0	0	0.2	0	0	0	0	0
Cs. impatiens	0	0	0	0.2	0	0	0	0
Cs. minnesotae	0	0	0.2	0	0	0	0	2.2
Cs. morsitans	0	0.1	2.7	3.6	0.1	0	0.5	7.7
Cx. pipiens	0	0	0	0.6	0	0	0	0
Cx. territans	0	0	9.7	79.5	7.4	0	0	0
Oc. abserratus	0	0	0	0	0	0	0.1	11.4
Oc. canadensis	0	1.5	13.5	26.5	39.1	0.8	3.6	59.3
Oc. cantator	0	0	0	0	0	0	0	0.4
Oc. communis	0	0	1.0	82.9	34.3	1.8	0.5	0
Oc. decticus	0	0	0	0	0	0	0.4	1.1
Oc. diantaeus	0	0	0	3.6	0	0	0	0
Oc. excrucians	0	0	0.2	0.2	1.0	0	0	2.0
Oc. fitchii	0	0	0.3	1.4	0.8	0	0	0
Oc. implicatus	0	0	0	2.1	3.1	0	0	0
Oc. intrudens	0	0	0	11.7	0	0	0	0
Oc. pionips	0	0	0.2	0.6	1.2	0	0	0
Oc. provocans	0	0	0.1	0.2	5.1	0	0	0
Oc. pullatus	0	0	0	0	0	0	0	0
Oc. punctor	0	0.8	14.8	9.2	14.4	2.2	3.2	54.8
				ADULTS				
n	6	43	15	84	18	8	54	194
Ae. cinereus	0	1	0	58	11	10	14	9
An. earlei	0	0	0	4	0	1	0	0
Cq. perturbans	0	14	120	290	74	53	116	452
Cs. impatiens	1	2	2	1	0	0	1	0

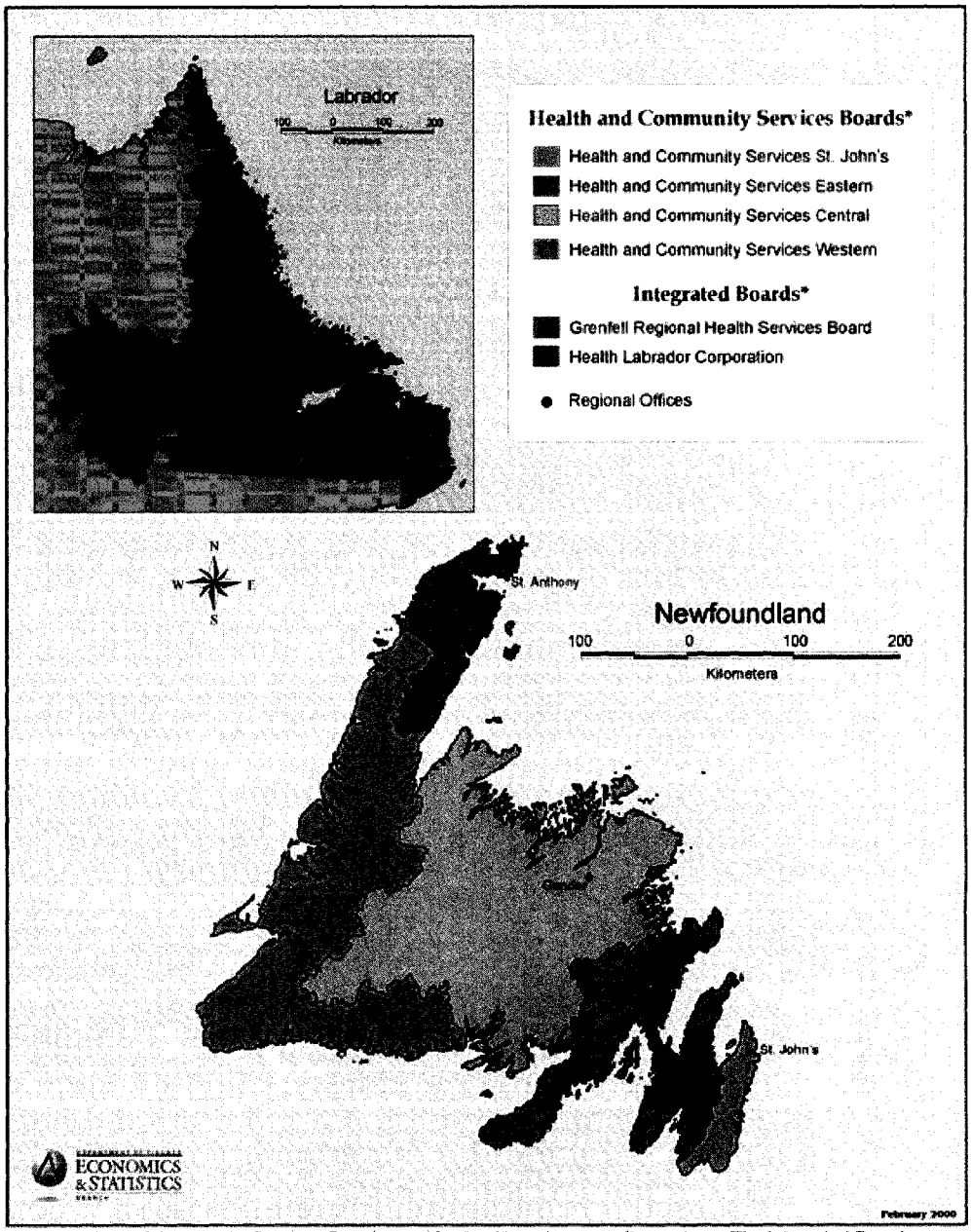
Table 8.2 continued

	Labrador	Western Parks	Corner Brook	Deer Lake	Cormack	Central Parks	Eastern Parks	St. John's
				ADULTS				
n	6	43	15	84	18	8	54	194
Cs. melanura	0	0	0	0	0	0	5	5
Cs. minnesotae	0	12	27	18	0	0	19	50
Cs. morsitans	6	26	0	31	3	16	39	248
Cx. pipiens	0	0	3	136	2	0	0	0
Cx. territans	0	0	0	0	0	0	1	0
Oc. abserratus/punctor	1	39	3	25	10	23	287	275
Oc. canadensis	0	162	20	158	32	32	323	618
Oc. cantator	0	1	0	0	0	1	54	1
Oc. communis	0	9	8	154	138	9	51	10
Oc. decticus	0	0	0	1	0	0	4	0
Oc. diantaeus	0	1	0	177	0	0	8	0
Oc. excrucians	3	2	1	189	13	2	15	104
Oc. fitchii	0	1	0	99	3	0	0	0
Oc. implicatus	0	0	0	1	0	1	0	0
Oc. intrudens	0	0	0	58	0	0	8	0
Oc. nigripes	1	0	0	0	0	0	0	0
Oc. pionips	1	0	0	0	0	0	0	0
Oc. provocans	0	0	0	11	2	0	0	0
Oc. pullatus	0	0	0	82	1	0	0	0

Table 8.3 Total larval abundances of mosquito species collected on the Island of Newfoundland, 2004-2005.

	Western Parks	Corner Brook	Deer Lake	Cormack	Central Parks	Eastern Parks	St. John's
n	4	80	210	110	4	8	262
Ae. cinereus	6	5	42	51	8	19	23
An. earlei	0	1	0	0	0	0	0
Cs. impatiens	0	0	1	0	0	0	0
Cs. minnesotae	0	1	0	0	0	0	11
Cs. morsitans	1	15	24	1	0	3	39
Cx. pipiens	0	0	3	0	0	0	0
Cx. territans	0	55	450	73	0	0	0
Oc. abserratus	0	0	0	0	0	1	84
Oc. canadensis	14	75	168	200	8	35	303
Oc. cantator	0	0	0	0	0	0	2
Oc. communis	0	5	428	172	9	7	0
Oc. decticus	0	0	0	0	0	6	6
Oc. diantaeus	0	0	18	0	0	0	0
Oc. excrucians	0	1	1	5	0	0	14
Oc. fitchii	0	2	8	5	0	0	0
Oc. implicatus	0	0	14	16	0	0	0
Oc. intrudens	0	0	60	0	0	0	0
Oc. pionips	0	1	3	6	0	0	0
Oc. provocans	0	2	1	27	0	0	0
Oc. punctor	6	80	68	76	13	32	341

8.2 Appendix 2: Health and community services boards of Newfoundland and Labrador.



* The Health and Community Services Boards provides health and community services. The Integrated Boards provide institutional, and health and community services.

Figure 8.1 A map of Newfoundland and Labrador outlining the health and community services boards.